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# Cancer Treatment Reports

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**9.** The methods of statistical analysis should be described in detail sufficient that a knowledgeable reader could reproduce the analysis if the data were available.

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**From* Methodologic Guidelines for Reports of Clinical Trials (Cancer Treat Rep 69:1-3, 1985).

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**791** ANNOUNCEMENTS

# Interaction of Etoposide and Cisplatin in an In Vitro Tumor Model[1,2]

**Ralph E. Durand* and James H. Goldie[3]**

**Some clinical data suggest that etoposide and cisplatin may be synergistic against certain types of tumors. Since studies of the process are both easier and more precise in laboratory systems, we have used V79 spheroids as a model to quantify the interaction of the drugs in cycling and noncycling, aerobic, and hypoxic cell subpopulations. The degree of interaction between etoposide and cisplatin in this system, quantified using the median-effect analysis, was highly dependent on the overall treatment toxicity, and to a lesser extent, the subpopulation of cells assessed. Synergism was demonstrated for relatively modest (1 decade) cell kills achievable at clinically relevant drug doses; increasing the toxicity of the combination treatment by increasing the cisplatin level produced a greater relative gain in the noncycling and hypoxic cells than in the aerobic, cycling cells of the spheroids. [Cancer Treat Rep 71:673-679, 1987]**

The antineoplastic agents cisplatin and etoposide (VP-16) each have significant therapeutic activity against a variety of experimental and clinical neoplasms (1-3). The demonstration by Schabel et al (4) of synergism between the two compounds prompted a number of clinical trials using the combination of VP-16 and cisplatin. Significant clinical activity of the combination has been shown in a variety of human malignancies, with at least some studies strongly suggesting a greater than additive effect (5-9). This would indicate that the synergism demonstrated in experimental systems may also occur at least in some clinical neoplasms as well.

True synergistic combinations in clinical cancer chemotherapy are rare, and therefore it would appear important to attempt to ascertain the biological and molecular basis of the synergism between VP-16 and cisplatin and to attempt to better define such questions as optimal dose ratio and schedule. In this report, we present data evaluating the cytotoxic effects of VP-16 and cisplatin alone and in combination against an in vitro multicell spheroid tumor model, Chinese hamster V79-171B spheroids, which display many tumor-like properties (10,11). The growth fraction of cells in spheroids decreases with time (12), concurrent with karyotypic instability (13) and the development of large numbers of noncycling cells. Nutrients and oxygen must diffuse into the spheroid from the periphery, so tumor-like central necrosis and hypoxia spontaneously develop in larger spheroids (14,15).

Analysis of interactions between drugs (and/or other biologically active agents) is complicated by a lack of universally accepted terminology and methodology (16). Since agent interactions are a function of the level of effect observed, we feel it is critical to analyze combination treatments over a range of effect levels. As a consequence, the studies reported here were designed to be analyzed by the "median-effect" principles described by Chou and Talalay (17); we have adopted their terminology throughout this report. Similarly, cytotoxicity of single or combined agents can be assessed using a number of different endpoints with these spheroids (18). We define viability here as clonogenic capacity of disaggregated single cells from the treated spheroids. Additionally, we have developed techniques of staining and fluorescence-activated cell sorting to selectively recover cells from *known* depths within the spheroid following drug exposure (15,19,20), thus permitting interactions to be studied in differing cell subpopulations.

Our results suggest therapeutically significant synergism between VP-16 and cisplatin, and that the synergism was more marked at progressively deeper levels within the spheroid when an increased level of cisplatin was used. The degree of synergism achieved in the more rapidly cycling external cells was, however, less strongly influenced by the dose ratios of the two agents.

## MATERIALS AND METHODS

### Cell Line and Culture Techniques

Chinese hamster V79-171B lung cells were used exclusively in these studies. These cells were routinely maintained as monolayers on plastic Petri dishes with

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[3] British Columbia Cancer Research Centre and the Cancer Control Agency of British Columbia, Vancouver, Canada.

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biweekly subcultivation in Eagle's minimal essential medium supplemented with 10% fetal calf serum (14,15). To initiate spheroid growth, asynchronous cells were removed from the plastic growth dishes by trypsinization. Spinner culture flasks (100 ml) were then inoculated with $10^6$ cells in 70 ml of minimal essential medium plus 5% serum and were stirred at 180 rpm; the gas phase contained 5% $CO_2$ in air. During the growth of the spheroids, the medium was first changed at Day 3; spheroids were transferred into 250-ml flasks at Day 4 or Day 5 and fed daily thereafter (spent medium was completely removed and replaced with fresh medium).

### Drugs and Treatment

The clinical formulation of VP-16 was used (Vepesid, Bristol Laboratories of Canada, Toronto, Ontario), and the desired concentration was added directly to the normal growth medium of the spheroids. Similarly, the clinical formulation of cisplatin (Bristol Laboratories) was diluted into spheroid growth medium as required. All drug exposures were for 2-hour intervals under normal growth conditions.

### Cell Sorting Procedures

Flow cytometry and fluorescence-activated cell sorting utilized a dual-laser Becton-Dickinson FACS-440. Spheroids were stained with Hoechst 33342 at 2 $\mu$M by adding the dye directly to the drug-containing flask for the final 20 minutes of drug exposure. At the end of the exposure, excess drug and stain were removed by aspiration after allowing the spheroids to sediment to the bottom of a collection tube. After three washes, the spheroids were reduced to a single-cell suspension by using 0.25% trypsin at 37°C for 10–12 minutes with continuous agitation. The stained, disaggregated single-cell suspension was then resuspended in at least three volumes of growth medium to one of trypsin and maintained at 4°C during the sorting procedure (typically $< 1$ hour).

The primary laser was operated at 400 mW and 488 nm with forward scatter and 90° scatter signals monitored. The ultraviolet laser was operated at 40-mW power with the 350- to 360-nm lines, and the Hoechst emission was monitored through a 449 ± 10-nm bandpass filter. The cell population was defined on the basis of the forward scatter signal; the resulting signals from the stain and 90° scatter were processed through matched logarithmic amplifiers, and the ratio of these signals used to generate a "stain concentration" profile, which was integrated to establish ten windows of equal cell numbers (15). The actual number of cells desired per Petri dish and the number of duplicates were preprogrammed, and the FACS was essentially utilized as a micromanipulator to deposit *known* cell numbers into "sort" tubes, which were then poured and rinsed into growth dishes (21).

Experiments to assess the toxicity of the Hoechst 33342, alone or in combination with VP-16 and/or cisplatin, were negative. However, selective interactions in a subpopulation of cells cannot be identified by measuring the entire population, yet the subpopulations cannot be studied without staining and sorting. Thus, our controls were to use either drug or the combinations at two to three levels of toxicity, and the stain concentration was then escalated to determine the level at which additional killing occurred. At least tenfold higher Hoechst 33342 concentrations than those typically used were required for any additional cell killing.

### Analysis of Interactions

As previously stated, we adopted the analytical procedures recommended by Chou and Talalay (17). The analysis is based on survival (S) being related to dose (D) according to the median-effect equation:

$$(1 - S)/S = (D/D_m)^m,$$

where m is the sigmoidicity of the curve, and $D_m$ is the median dose (which produces 50% survival). A log transform of the equation simplifies solution for the constants; resulting parallel curves indicate treatment agents can be added by dose, and the dose giving survival S is:

$$D = D_m[(1 - S)/S]^{1/m}.$$

The fractional effect (f1) due to Drug 1 in a two-drug scheme then varies with its concentration (C1):

$$f1 = C1/(C1 + C2).$$

Thus, the combination index (CI) can be defined:

$$CI = \frac{(D12)(f1)}{D1} + \frac{(D12)(f2)}{D2} + \frac{(D12)(f1)(D12)(f2)}{(D1)(D2)},$$

where the last term is required only if the agents are nonexclusive (m is greater for the combined treatment than for either single agent). In essence, the CI is the ratio of the combination dose to the sum of the (isoeffective) single-agent doses; consequently, $CI < 1$ shows potentiation (synergism) and $CI > 1$ indicates antagonism (protection).

## RESULTS

Cisplatin is an effective single agent in V79 cells, although the response of cells exposed to the drug while in situ in a spheroid is quite different than that of the *same* cells if disaggregated to single cells just prior to drug exposure (22). A more detailed, composite response from several experiments is shown for selected drug concentrations in figure 1, where drug activity (toxicity) was plotted as a function of depth into the spheroid (based on the measured survival of the sorted, recovered cell subpopulations).

The external, cycling cells of the spheroids were most sensitive to cisplatin, with less toxicity at greater depths

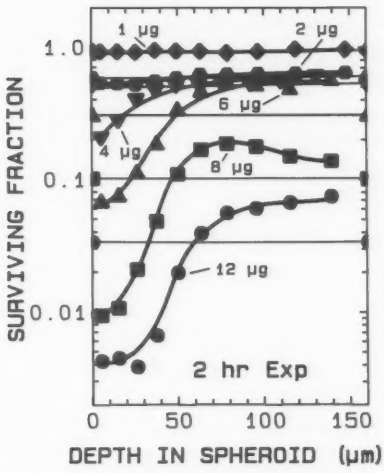


FIGURE 1.—Survival of spheroid cells exposed to the indicated quantities of cisplatin per milliliter, as a function of location in the spheroid at the time of drug treatment. Horizontal lines indicate the average response; curves from several experiments are included.

within the spheroids. Since the differential toxicity was *not* retained when sorted single cells were exposed to the drug (22), the differential apparently does not reflect inherent drug resistance, but rather, decreased drug efficacy due either to poor penetration or to the microenvironment in the spheroids. Interestingly, *more* toxicity at a given cisplatin dose is always observed in intact spheroids relative to exposure under dissociated conditions (22).

Similar patterns of VP-16 toxicity were found (fig 2), although some interesting differences are also noted. The differential response was much greater, with little killing of internal cells even for extremely toxic (to external cells) drug concentrations. Unlike cisplatin, VP-16 survival profiles for cells separated prior to drug exposure were qualitatively similar to those of cells exposed in intact spheroids (data not shown); however, about a twofold increase in drug concentration was required to achieve similar levels of toxicity in spheroids as in the separated cells (again contrasting to the reduction for cisplatin). For VP-16, the differential response is thus apparently reflecting a difference in inherent drug sensitivity through the spheroid.

The resistance of selected subpopulations of cells from the spheroids is shown in a more traditional dose-response format in figure 3 for the outermost 10% of the cells, the innermost 10%, and a fraction selected from midway through the spheroids. The greater differential response between the inner and outer cells for VP-16 is perhaps shown more clearly in this format; it is also interesting to note that the data were much "tighter" for the multiple VP-16 determinations than for the cisplatin determinations, despite the fact that many were simultaneously performed on the same spheroid populations. This perhaps suggests less influence of environmental factors (spheroid size, density in the growth flask, etc) on the net response to VP-16.

Combination treatments with VP-16 and cisplatin (fig 4) produced considerably more toxicity than equivalent (summed) concentrations of either single agent (compare, for example, the 6 µg/ml of VP-16 + 6 µg/ml of cisplatin curve in fig 4a with any curves of figs 1 or 2). Two combinations were evaluated: equal drug levels (on a microgram-per-milliliter basis) in Panel a, and a five-fold reduction in cisplatin concentration as in Panel b. Several features were noteworthy. First, the increased resistance of internal cells after single-drug exposure was maintained in the combinations. Additionally, and as would be expected on the basis of the single-agent toxic effects presented, more killing was observed for a 24-µg/ml total exposure consisting of equal doses (12 µg/ml) of VP-16 and cisplatin than for 20 µg/ml of VP-16 plus 4 µg/ml of cisplatin. Although difficult to quantify, it appeared that the increased effectiveness of the combination treatments was also relatively greater on the innermost cells.

The dose-response curves generated can be illustrated as previously shown in figure 3 or can be analyzed in terms of the median-effect model (briefly discussed in the Materials and Methods section). For unambiguous use of the median-effect principle to calculate combination indices, a reasonable fit of the experimental data to the model is required, as is parallelism of the derived dose-response curves plotted in that manner (17). Sample curves for the transformed median-effect equation are shown in figure 5, with the corresponding data for the 1:1 combination of VP-16 and cisplatin; the concave-upward nature of the latter response was indicative of

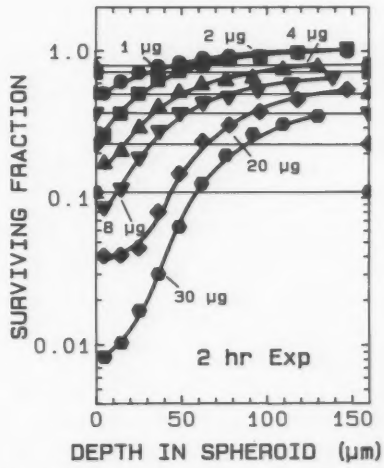


FIGURE 2.—Survival of spheroid cells exposed to the indicated quantities of VP-16 per milliliter, as a function of location in the spheroid at the time of drug treatment. Horizontal lines indicate the average response; representative curves from several experiments are again included.

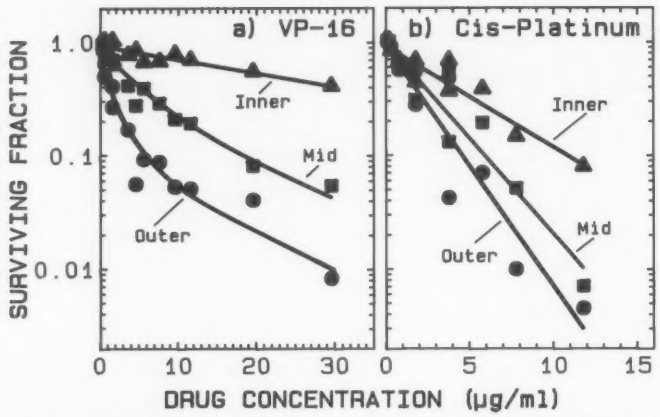


FIGURE 3.—Survival of selected subpopulations of cells from spheroids exposed to the indicated quantities of VP-16 (Panel a) or cisplatin (Panel b) per milliliter, as a function of drug concentration present for the 2-hr exposure. As in the previous figs, data from several experiments are shown; the curves represent the outermost 10% of the cells from each spheroid population, the innermost 10%, and the fourth fraction sorted (a population about midway through the viable rim of cells of the spheroid).

nonexclusive interaction of the agents (17). It is immediately evident that the combination provokes a synergistic interaction when the data are presented in this manner, since the plot (fig 5) is essentially effect as a function of dose and the combination-treatment data (filled symbols) lie well above the single-agent curves. Additionally, presentation of the data in this manner allows an immediate assessment of reproducibility, since each point on the combination curve came from a single representative experiment, whereas the single-agent curves included data from this experiment as well as several other independent determinations.

Similar median-effect relationships were determined for each cell subpopulation and exposure situation and the combination indices calculated. Since these values were continuously varying throughout the spheroid, a fairly simple interpolation (if sort fraction rather than depth in the spheroid was used to linearize the interpolation increments) leads to figures 6 and 7, where the combination index (CI) is shown as a function of sur-

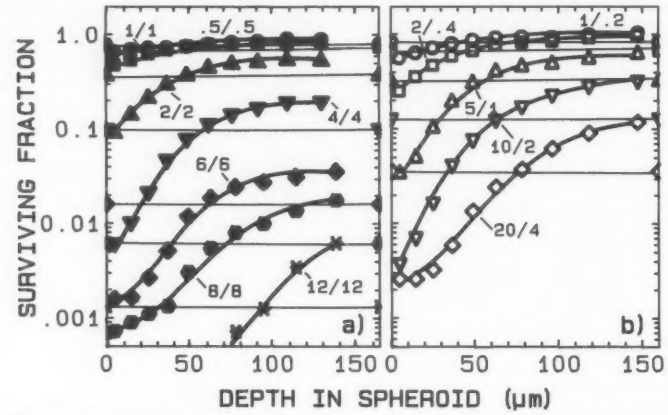


FIGURE 4.—Survival of spheroid cells exposed to two combinations of VP-16 and cisplatin, plotted as a function of location in the spheroid at the time of drug treatment. In Panel a, equal drug levels (μg/ml) were used; Panel b shows results for VP-16 concentrations 5-fold higher than cisplatin. Horizontal lines again indicate the average response of all cells at risk.

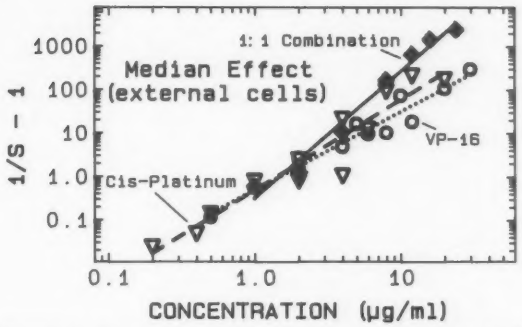


FIGURE 5.—Survival data for the outermost 10% of the spheroid cells are plotted in the format required to deduce the constants of the median-effect equation (*see* Materials and Methods section). Note that the curves for VP-16 and cisplatin are essentially parallel, and the closed symbols (combinations at a 1:1 ratio) show greater effectiveness (lie above the single-agent curves) at higher doses. All curves displayed regression lines with correlation coefficients > 0.9 (as required to validate this analysis, eg, ref 17).

vival level and of cell position in the spheroid. In both figures, synergism was clearly indicated (CIs < 1.0) if effective levels of the agents were used, and progressively more interaction occurred as toxicity (drug concentration) increased. It should also be noted that the median-effect analysis provides a conservative estimate of synergism: low-dose antagonism is *overestimated* and high-dose synergism is *underestimated* by representing the (concave-upward) combination data (eg, fig 5) with the (linear) median-effect model.

## DISCUSSION

These data clearly indicate that combination treatments of V79 spheroids with VP-16 and cisplatin can be synergistic, and perhaps more importantly, that the agents, at appropriate concentrations, were synergistic in *all* subpopulations of the spheroids. Our hope in the initial design of this study was to estimate whether one of the two combinations was likely to be more effective in the clinic (an ongoing protocol in our institution is comparing 3-week sequences of daily treatments with 100 mg/m$^2$ of VP-16 + 20 mg/m$^2$ of cisplatin to 100 mg/m$^2$ of VP-16 daily + 100 mg/m$^2$ of cisplatin once weekly only). To achieve cure of a neoplasm, eradication of the most resistant tumor stem cell population is necessary; unfortunately, it is not immediately evident which subpopulation of cells in the spheroid best represents that population.

To illustrate this point, suppose, for example, that the 100:20 clinical combination produced a minimal cell kill of 50% (in the spheroids, fig 4b, the necessary dose was

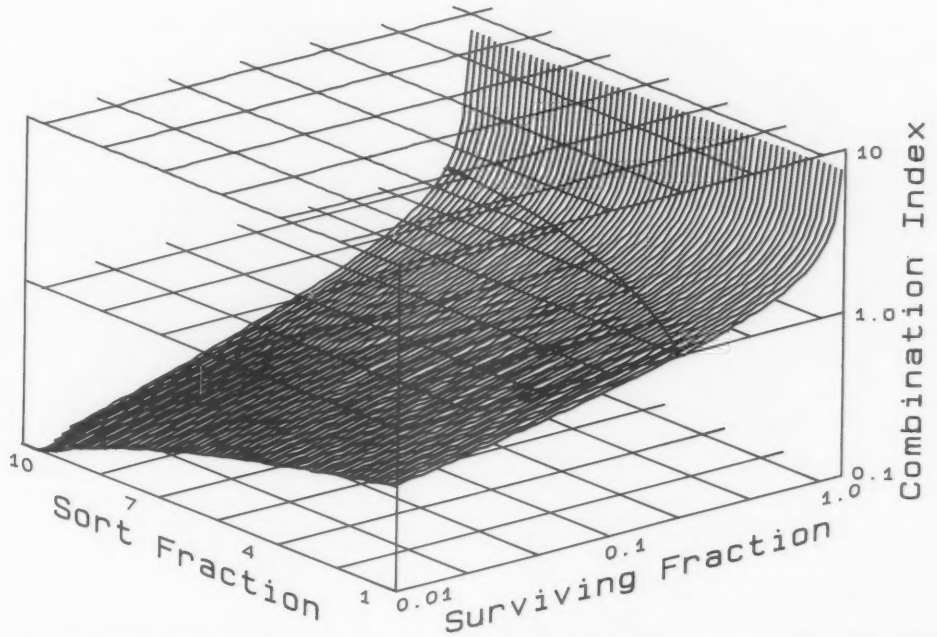


FIGURE 6.—Derived CI for VP-16 and cisplatin exposures at a 1:1 ratio is plotted as a function of effect level (surviving fraction) and depth in the spheroid (sort fraction, where fraction 1 is the outermost cells). Reference planes are superimposed on the response surfaces at CI = 1.0 (additive response) and 10-fold greater and lesser levels. Note that CI < 1.0 indicates synergism between the agents; the actual value of CI is essentially the dose ratio of the combination to single agents for the shown level of effect.

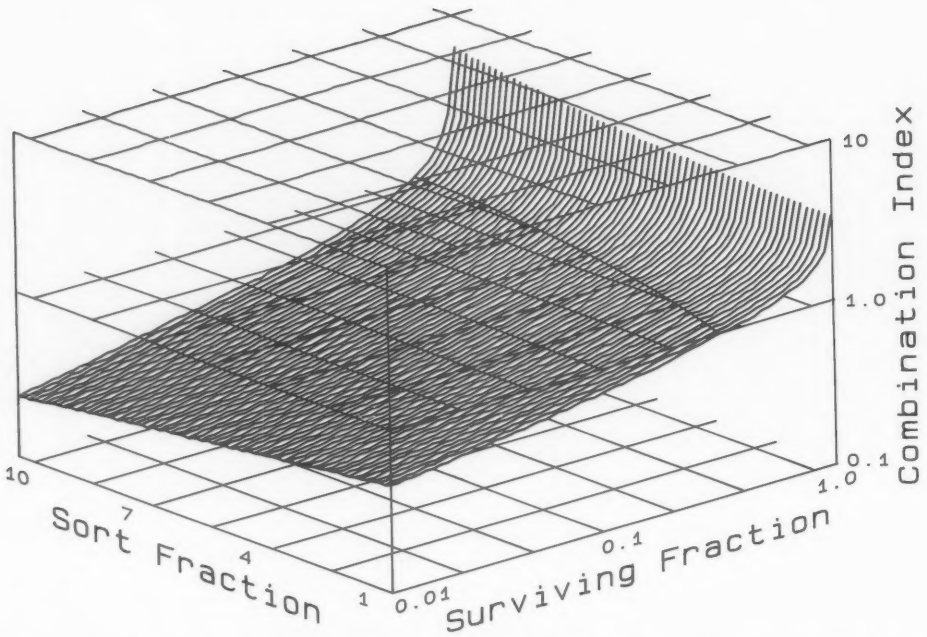


FIGURE 7.—Derived CI for VP-16 and cisplatin exposures at a 5:1 ratio is plotted as in fig 6. Note that this plot presents the CI as a function of level of effect; greater drug doses are required to reach any surviving fraction than in fig 6.

10 μg/ml of VP-16 + 2 μg/ml of cisplatin); five such treatments would then lead to a surviving fraction of 0.03 in the most resistant cells. The proportionately larger effect of the 100:100 combination (ie, 10 μg/ml of each agent in spheroids) would lead to roughly 1% survival after the first treatment, and the four subsequent VP-16 treatments at the same level (0.7 survival, fig 2) would reduce survival by another factor of 0.25, resulting in about 1-decade *additional* cell kill for the combination with the higher cisplatin concentration.

Now consider the same treatments, but with the assumption that the internal (drug-resistant) cells of the spheroid, which are known to be quiescent (nonproliferating) at the time of treatment, are in fact nonclonogenic in situ in the spheroid (or, by analogy, in the tumor). Under these conditions, the response of the external cells is of prime importance; the same 100:20 combination would lead to daily decreases in that population by factors of 0.03, resulting in 8 decades of cell kill per week. Approximately 4 decades of cell kill would be expected by the first 100:100 combination treatment, with one additional decade daily by the VP-16, for an essentially *identical* final response. Very different conclusions are thus reached depending on the choice of the target cell population; the situation is further complicated in that this analysis does not allow any changes in cell subpopulations during the treatment, which is an obvious oversimplification since each treatment clearly selects for resistant cells.

It must also be emphasized that the resistant cells present in the interior of the spheroid (as identified by single treatments) are likely to be different from genetically resistant cells that are selected out by multiple courses of treatment. Whether the biochemical resistance mechanisms have anything in common is not known, but they certainly cannot be assumed to be identical. Thus, the question of therapeutic activity of this combination against genetically resistant cells must remain open.

It is interesting to note that the CI analysis of figures 6 and 7 suggests somewhat different interactions for the two combinations: at treatments which produce 2 decades of toxicity, a relatively constant gain is expected through the spheroid in the case of the 5:1 VP-16 to cisplatin ratio, whereas the 1:1 ratio led to an increased gain in the innermost cells (which is likely to be of some relevance if those cells parallel clinical resistance). Some care is required in assessing the data presented in figures 6 and 7, however, since *increased* drug concentrations are required to reach the same isoeffect level in the internal regions of the spheroids (a similar result was obtained for both drug dose ratios; fig 4).

To circumvent this problem, the observed CI in spheroids has been replotted in figure 8 as a function of the total drug dose administered. Only two cell subpopulations are indicated, the innermost and the outermost 10% of the cells. Similar conclusions are forthcoming from the data presented in this manner: a greater inter-

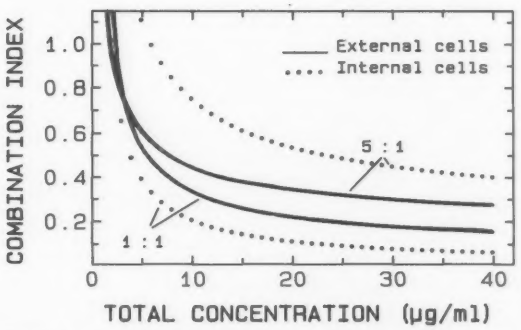


FIGURE 8.—Derived CI for VP-16 and cisplatin is plotted as a function of cumulative drug dose for the innermost and outermost cell subpopulations of the spheroids. Upper curves indicate the interaction when $^1/_6$ of the indicated total drug dosage was cisplatin, and the lower curves indicate the response for an equal drug ratio. Note that more synergism in the innermost cells was observed only for the latter protocol.

action was seen when cisplatin was used at the same concentration as VP-16 than when used at 1/5 the dosage, and further, the relative interaction in the internal compared to external cells (in spite of less net killing at a particular dose) was greater for the higher cisplatin ratio. Perhaps of even more significance, considerable synergism was observed in spheroids at clinically achievable drug levels (the 2-10-µg/ml range), since plasma levels of at least this magnitude have been reported in patients for both VP-16 (23) and cisplatin (24).

It remains to be established of course whether any clinical synergism that may exist between these two agents is in any way related to the observations in this report. Similarly, the molecular mechanism(s) of the interaction remains to be elucidated; in view of the known ability of VP-16 to create DNA strand breaks (25) and the dependence of cisplatin binding on the local DNA structure or integrity (26), the suggestion that synergism may depend on this mechanism (26) is, however, quite compatible with our results. Though the immediate applicability of this type of data to clinical treatment is not clear-cut, we hope that our demonstration of synergism of VP-16 and cisplatin in a relevant tumor model and our data suggesting that different degrees of interaction occur in different cell subpopulations and at different dose ratios of the two agents will provoke further study of a promising clinical combination.

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# Concurrent Cisplatin and Etoposide With Radiotherapy in Locally Advanced Non-Small Cell Lung Cancer[1,2]

**Gregory G. Friess,* Madhara Balkadi, and Walter H. Harvey[3,4]**

**Twenty consecutive patients with newly diagnosed or locally recurrent non-small cell lung cancer limited to the chest were treated with concurrent radiotherapy and cisplatin plus etoposide. No patient had received prior chemotherapy or radiotherapy. Sixteen of 20 patients (80%) responded to the treatment. Median survival has not been reached but is > 13.5 months. Twelve patients have relapsed at a median of 10 months after starting therapy (range, 4–12 months), with only one initial relapse within the radiotherapy port. Hematologic toxicity was mild, with four of 52 wbc count nadirs < 1000/mm$^3$. Esophageal toxicity was also mild, with all patients maintaining oral intake throughout therapy. We conclude that simultaneous radiotherapy, cisplatin, and etoposide can be safely administered in locoregional non-small cell carcinoma of the lung. A prospective trial of this regimen versus radiotherapy alone is warranted. [Cancer Treat Rep 71:681–684, 1987]**

Locally advanced non-small cell carcinoma of the lung (NSCCL) of an unresectable nature is a frequent problem, with approximately 35,000–40,000 patients detected annually in the United States (1). This group, commonly staged as stage III $M_0$, is usually treated with radiotherapy to the primary tumor and draining nodal basins. This has yielded approximately 6% long-term survivals (2,3). The role of chemotherapy is less clear but has been extensively investigated (4,5). We report here a trial of concurrent cisplatin and etoposide chemotherapy with daily radiotherapy in previously untreated patients with locally advanced unresectable NSCCL.

## MATERIALS AND METHODS

From December 1983 to January 1986, 23 adult patients with histologically proven NSCCL were registered in this study. Eligibility criteria for this study included recurrent or unresectable disease limited to the thoracic cavity, no prior chemotherapy or radiation therapy, normal serum creatinine, adequate pulmonary reserve to tolerate planned radiotherapy, wbc count > 4000/mm$^3$, platelet count > 100,000/mm$^3$, and informed written consent. All patients had computed tomography of the chest and negative bone scans prior to institution of treatment. Normal liver chemistries or liver imaging was also required. All patients were staged at the time of beginning therapy according to criteria published by the American Joint Committee on Cancer (6). On review following registration, three patients were excluded from analysis. Two of these patients were demonstrated to have metastatic disease beyond the thoracic cavity prior to the institution of therapy and one patient had undergone resection of all known tumor prior to registration. These three patients are included for toxicity analysis for the first course of chemotherapy and radiation therapy but are excluded from survival and response analysis.

Following registration, all eligible patients received external-beam radiotherapy at 180 cGy daily to a total dose of 4500 cGy covering the entire gross tumor volume, ipsilateral and contralateral hilar nodes, subcarinal nodes, superior mediastinal nodes, and supraclavicular nodes. A 1.5-cm margin was incorporated into the initial target volume. A boost dose to the area of gross residual tumor was added with chemotherapy; total dose was 5500–6100 cGy. All patients were treated with a 6-meV linear accelerator with a target distance of 100 cm (source to skin). Specially made custom cerrobend blocks were utilized in most patients with appropriate use of compensators and bolus materials in specific instances to deliver a homogeneous dose. The spinal cord was routinely shielded after 4000 cGy and maximum dose to the heart was limited to 5000 cGy.

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[2] The opinions or assertions contained herein are the private views of the authors and are not to be construed as reflecting the views of the Department of the Army or the Department of Defense.

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On Day 1 of radiotherapy, concurrent chemotherapy was begun consisting of cisplatin at 50 mg/m$^2$ iv on Days 1 and 8 with etoposide at 50 mg/m$^2$ iv on Days 1–5. Saline hydration was given prior to each dose of cisplatin to maintain adequate urine output. Metoclopramide and dexamethasone were utilized as antiemetics. Chemotherapy was repeated at 28-day intervals during radiotherapy, thus giving two courses of chemotherapy during the 42-day course of irradiation. Two further courses of chemotherapy were then offered to responding patients after completion of radiotherapy.

Wbc counts, platelet counts, and serum creatinine values were assayed frequently during concurrent therapy. Chemotherapy was delayed by 1 week for wbc counts $< 3000/mm^3$, absolute neutrophil counts $< 2000/mm^3$, or platelet counts $< 100,000/mm^3$ at the time treatment was due. Radiotherapy was delayed for myelosuppression of wbc count $< 2000/mm^3$, absolute neutrophil count $< 1500/mm^3$, or platelet count $< 100,000/mm^3$ until such time as hematologic recovery was attained.

Patients were assessed for response at 2 and 4 months after starting therapy in the absence of evidence of disease progression. Standard definitions of response were used (7). Partial response was defined as a $\geq 50\%$ reduction in the sums of the products of the perpendicular diameters of all measurable lesions for $> 4$ weeks. Complete response was defined as the absence of all measurable disease for $> 4$ weeks. Complete responders were verified histologically when possible. At the completion of therapy, patients were followed for time to disease progression and survival.

## RESULTS

Twenty evaluable patients (ten men and ten women) were registered in this study. Median age was 57.5 years (range, 34–72). Histologic diagnosis consisted of squamous cell carcinoma in five patients, adenocarcinoma in nine patients, and large cell/undifferentiated carcinoma in six patients. Thirteen of 20 patients had T3 lesions, and ten of 20 patients had histologically positive mediastinal, cervical, or supraclavicular lymph nodes. Full TNM staging and reasons for unresectability are shown in table 1. Sixteen of 20 patients responded to therapy: four complete responses and 12 partial responses. Complete responses were shown radiologically in three patients and at autopsy in the fourth. Three of the remaining four patients had stable disease or minor response. The final patient had disease confined to bilateral mainstem bronchi and declined second bronchoscopy to determine response. This patient is considered to have stable disease. All four nonresponding patients were considered treatment failures. Median survival has not yet been reached but is $> 13.5$ months, with a range of 2–30+ months. To date, 12 patients have relapsed. This recurrence occurred at a median of 10 months from starting therapy, with a range of 4–18 months. Primary site of relapse was within the radiation therapy port in only one patient, with the remaining 11 patients relapsing initially outside the irradiated field. All sites of first relapse are shown in table 1.

Toxicity is shown in table 2. No treatment-related deaths occurred. In four of 52 chemotherapy courses, wbc count nadir dropped to $< 1000/mm^3$ (7.7%). The

TABLE 1.—Patient characteristics

| Patient No. | Age (yrs) | Sex | TNM staging | Histology | Response* | First-site relapse | Survival (mos) | Unresectable due to: |
|---|---|---|---|---|---|---|---|---|
| 1 | 62 | F | T3Nx | Squamous cell carcinoma | CR | | 2 | Proximal tumor with left lung collapse |
| 2 | 37 | F | T3N2 | Adenocarcinoma | PR | Bone | 15 | Tracheal involvement + cervical node |
| 3 | 62 | M | T3Nx | Adenocarcinoma | Stable | Bone | 22 | Recurrent after resection |
| 4 | 72 | M | T3N2 | Squamous cell carcinoma | PR | Local | 14 | Pericardial and atrial invasion |
| 5 | 53 | M | T3Nx | Adenocarcinoma | PR | Brain | 30+ | Superior vena cava syndrome |
| 6 | 44 | M | T3N0 | Large cell carcinoma | CR | Soft tissue | 13 | Tracheal involvement |
| 7 | 66 | M | T3Nx | Squamous cell carcinoma | PR | Liver | 12 | Proximal involvement |
| 8 | 55 | F | T3Nx | Adenocarcinoma | Stable | Lung/bone | 6 | Recurrent after resection |
| 9 | 61 | M | T3Nx | Large cell carcinoma | — | | 18+ | Bilateral mainstem tumors |
| 10 | 66 | F | T2N2 | Large cell carcinoma | PR | Lung | 19 | Histologic + mediastinal nodes |
| 11 | 57 | M | T3Nx | Squamous cell carcinoma | PR | Soft tissue | 11 | Recurrent after resection |
| 12 | 34 | F | T2N2 | Large cell carcinoma | CR | | 21+ | Histologic + mediastinal nodes |
| 13 | 58 | M | T2N2 | Undifferentiated carcinoma | PR | Liver | 13+ | Histologic + mediastinal nodes |
| 14 | 53 | M | T3N2 | Adenocarcinoma | PR | | 14+ | Histologic + mediastinal nodes |
| 15 | 61 | M | T3Nx | Adenocarcinoma | PR | | 13+ | Malignant pleural effusion |
| 16 | 65 | F | T2N2 | Squamous cell carcinoma | PR | Soft tissue | 10 | Histologic + mediastinal nodes |
| 17 | 56 | F | T3Nx | Adenocarcinoma | Stable | | 9+ | Recurrent after resection |
| 18 | 48 | F | T2N2 | Adenocarcinoma | PR | | 9+ | Incomplete resection |
| 19 | 52 | F | T2N2 | Undifferentiated carcinoma | CR | | 7+ | Histologic + cervical node |
| 20 | 54 | F | T2N2 | Adenocarcinoma | PR | Bone | 5 | Histologic + supraclavicular node |

*CR = complete response; PR = partial response; and stable = stable disease.

TABLE 2.—Toxicity of concurrent cisplatin, etoposide, and radiotherapy

| Toxic effect | |
|---|---|
| Hematologic* | |
| Wbc count nadir, $mm^3$ ($n = 52$) | |
| ≥ 2000 | 37 (71) |
| 1999–1000 | 11 (21) |
| ≤ 999 | 4 (7.7) |
| Absolute neutrophil count nadir, $mm^3$ ($n = 46$) | |
| ≥ 1000 | 37 (80) |
| 999–500 | 5 (11) |
| ≤ 499 | 4 (9) |
| Platelet count nadir, $mm^3$ ($n = 52$) | |
| ≥ 100,000 | 48 (92) |
| 99,000–50,000 | 2 (3.8) |
| ≤ 49,999 | 2 (3.8) |
| Esophageal ($n = 20$)† | |
| None | 3 (15) |
| Grade 1 | 9 (45) |
| Grade 2 | 8 (40) |
| Grade 3 | 0 |

*Values = No. of courses (%).

†Values = No. of patients (%). Toxicity definitions are: Grade 1 = dysphagia or odynophagia not requiring treatment; Grade 2 = dysphagia or odynophagia requiring antacids or viscous lidocaine; and Grade 3 = dysphagia or odynophagia lasting > 14 days after treatment.

absolute neutrophil count nadir dropped to $< 1000/mm^3$ in nine of 46 courses (19.6%), with platelet count nadirs $< 100,000/mm^3$ being noted in four of 52 courses (7.7%). One episode of culture-negative febrile neutropenia occurred, which resolved with antibiotics. The same patient required a prophylactic platelet transfusion during the same febrile neutropenic episode, for a platelet count of $25,000/mm^3$. Esophagitis was mild to moderate, with eight of 20 (40%) patients reporting more than minimal symptoms. No patient required cessation of therapy or stopped oral intake due to esophagitis. Two patients experienced late esophageal dysfunction at 11 months posttherapy. This consisted of achalasia in one patient and stricture in another. Both patients were later found to have tumor encasing the esophagus as an etiology of their dysfunction. Nausea and vomiting occurred during 45 of 52 courses of treatment. This was in general transient and controlled through the use of previously described antiemetics. Renal toxicity was not encountered. There were no severe skin toxic effects or clinically significant pulmonary reactions seen. Infiltrates within the radiation field were visible on chest roentgenogram in most patients 4 months after starting therapy. One early death occurred on this study when Patient 1 had cardiopulmonary arrest on Day 50 of therapy. This patient had a prior history of episodic atrial fibrillation and death occurred shortly after reexpansion of a previously atelectatic left lung. At autopsy, no evidence of residual tumor was found. There was also no evidence of pulmonary embolism, myocardial infarction, infection, or other apparent cause of death.

Delays in radiotherapy were in general not excessive, with ten patients experiencing no delay and ten experiencing a delay of 1–17 days. Five of the delayed patients had treatment withheld for myelosuppression for 2, 3, 3, 5, and 5 days. Three patients were delayed at their own request for social reasons for 4, 9, and 17 days. The longest delay was 17 days due to alcohol-related illness. Two patients were delayed for 1 day to control cisplatin-induced vomiting.

## DISCUSSION

Standard therapy for unresectable locoregional NSCCL has been radiation therapy. In a large series of irradiated patients, the Radiation Therapy Oncology Group has reported a 46%–68% response rate and a median survival of 35–55 weeks (8). Relapse within the treatment field was the primary site of failure in 38%–64% (9). Prior attempts to potentiate radiation therapy by adding simultaneous chemotherapy have been made. Single chemotherapeutic agents have included 5-fluorouracil, cyclophosphamide, and vinblastine with little added benefit and significant added toxicity (10–12). More recently, cisplatin has been investigated as a potentiator with radiotherapy. Reimer et al (13) assessed concurrent radiotherapy and weekly cisplatin at doses ranging from 20 to 50 $mg/m^2$. Thirteen patients were treated, with 11 responses (seven complete and four partial) within the radiation port. No NSCCL patients were in this study. Schaake-Koning et al (14) assessed the toxicity of concurrent cisplatin and radiotherapy in 20 patients with inoperable locoregional NSCCL. They found the dose-limiting toxic effect to be vomiting at 35 $mg/m^2$ iv weekly of cisplatin and recommended a dose of 30 $mg/m^2$ iv weekly for further study. The radiotherapy employed was given in 300-cGy fractions daily × 10 days followed by a 2-week break, for a total tumor dose of 6000 cGy. Seventeen of 20 patients responded, with ten complete responders. Finally, Coughlin et al (15) used cisplatin prior to and during radiotherapy in 13 patients with locoregional squamous cell cancer of the lung. The dose of cisplatin was an initial 100-$mg/m^2$ iv bolus dose, with radiotherapy being delivered 21 days later. During the concurrent phase of radiation therapy, cisplatin was administered in 20-$mg/m^2$ doses weekly. Toxicity in this patient group was moderate, with all patients completing therapy. Six of 13 patients were alive 1 year after starting therapy.

Our study investigated the possibility of giving concurrent cisplatin and etoposide during radiation therapy of the primary tumor. This study was designed with the rationale of having the single best modality, that is radiotherapy, given initially to the bulk of tumor. Synergy between radiotherapy and chemotherapy administered simultaneously, as well as the early treatment of metastatic disease beyond the radiation therapy port, was perceived as a possible added benefit. Response to therapy occurred in 16 of 20 patients (80%), with four complete responders. Survival in our patients has not

reached a median value but is at least 13.5 months. Twelve patients have recurred at a median of 10 months since the initiation of therapy. Local tumor control was good, with primary relapse within the radiation therapy port in only one patient. One other patient had locoregional complications after an initial bony relapse. Toxicity of concurrent cisplatin and etoposide with daily radiotherapy was mild to moderate, with no treatment-related deaths. One death during treatment occurred, possibly due to cardiac dysrhythmia. Acute esophageal toxicity was notably minor in the study, in contrast to a previous study utilizing doxorubicin (16). All patients maintained oral intake throughout the cisplatin, etoposide, and radiation therapy phase of treatment. No patients received or required parenteral hyperalimentation. This lack of major esophageal toxicity during treatment could represent a significant benefit to patients treated in this fashion (16).

An important question in combined-modality studies of lung cancer is whether the radiotherapy can be given in a timely fashion. In our study, half of our patients encountered no delay in radiotherapy despite the concurrent administration of chemotherapy. Patients with a delay in therapy had a median delay of 5 days. Furthermore, the longest delays in therapy were for social reasons, unrelated to treatment of the underlying tumor.

In conclusion, we have shown the ability to safely administer cisplatin and etoposide with concurrent radiotherapy in limited stage III NSCCL. The true benefit and utility of this form of therapy remain to be determined in a prospective randomized trial comparing radiotherapy in this form of combined-modality treatment. We feel such a trial is warranted with these initial results.

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# Response to Tamoxifen and Fluoxymesterone in a Group of Breast Cancer Patients With Disease Recurrence After Cessation of Adjuvant Tamoxifen[1]

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**The response to tamoxifen or a combination of tamoxifen and fluoxymesterone was assessed in 54 postmenopausal breast cancer patients with recurrent disease. The patients had originally been entered in a randomized trial of 2 years of tamoxifen (40 mg daily), as an adjunct to primary surgery, versus no adjuvant endocrine therapy. The objective response rate (complete + partial) to the mentioned salvage endocrine therapies was significantly lower among patients from the tamoxifen group as compared to the controls (14% vs 54%; $P < 0.01$). Their median time to disease progression was also significantly shorter (4 vs 15 months; $P < 0.05$). Differences between the groups in regard to prognostic factors could not explain these differences. The lower response rate to these endocrine treatments was possibly one reason for the poorer survival outlook after relapse observed among the patients previously treated with adjuvant tamoxifen. An increased relapse-free survival achieved with adjuvant therapy may thus to some extent be offset by a poorer survival after relapse. [Cancer Treat Rep 71:685-688, 1987]**

Tamoxifen has been used in breast cancer as an adjunct to primary surgery in several controlled trials (1-6). Many investigators have reported a prolongation of the relapse-free interval, but few have observed a significant survival gain (2,6). The inconsistency between an increased relapse-free interval and no apparent survival gain could be due to short follow-up, for example, but could also be the result of a poorer survival outlook after relapse among patients treated with adjuvant tamoxifen as compared to the control patients. The aim of the present investigation was to determine if a decreased response to salvage endocrine treatment may have contributed to such a survival difference. The patients included in the study were obtained from a randomized trial of adjuvant tamoxifen in early breast cancer, which is being conducted in the Stockholm area.

## MATERIALS AND METHODS

A randomized trial of adjuvant tamoxifen in early breast cancer was initiated by the Stockholm Breast Cancer Study Group in 1976; the trial is still open. Until June 1983, 1159 postmenopausal breast cancer patients < 71 years of age were entered in the trial. After primary surgery, a total of 574 patients were randomized to 2 years of adjuvant tamoxifen (40 mg daily). The remaining 585 patients served as a control group and did not receive adjuvant endocrine therapy. A total of 368 patients had histologically verified axillary lymph node metastasis or a tumor diameter > 3 cm and were superrandomized to either postoperative radiotherapy or 12 courses of CMF (cyclophosphamide, methotrexate, and 5-fluorouracil) chemotherapy. Details of the trial design and preliminary results have been reported elsewhere (4). In short, the results indicated that adjuvant tamoxifen significantly reduced the number of treatment failures but no significant survival gain was observed. The end date for the current follow-up was June 30, 1985. The follow-up times thus ranged from 25 to 103 months, with a median of about 5 years. Less than 1% of the patients were lost to follow-up. Disease recurrence was confirmed when possible by biopsy. Bone and visceral metastases, however, were usually established on unequivocal radiological evidence.

Salvage treatment in case of recurrent disease was decided individually for each patient by the responsible clinician according to principles agreed upon by the Stockholm Breast Cancer Study Group. These principles implied that patients with a recurrence diagnosed during treatment with adjuvant tamoxifen were not eligible for salvage endocrine therapy. Consequently, in the tamoxifen group, only patients with recurrent disease diagnosed after 2 years could be included in the current study. Since the length of the disease-free inter-

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val is an important predictor of response to salvage therapy and survival after relapse, we also restricted our analysis of the control group to patients with recurrent disease diagnosed > 2 years after the initial randomization. The number of patients with recurrences after 2 years was 59 from the tamoxifen group and 68 from the control group. Clinical records and pathology reports were analyzed retrospectively. We then selected those patients who had at least one evaluable metastatic site and who received endocrine therapy as their first and only pharmacological relapse treatment. Thus, the final number of patients selected for the current investigation was 28 from the tamoxifen group and 26 from the control group (table 1). In these groups, the median follow-up times from diagnosis of the recurrence were 12 and 19 months, respectively.

According to accepted routines, endocrine salvage treatment was usually tamoxifen (40 mg daily). A few patients, however, were treated with fluoxymesterone or a combination of tamoxifen and fluoxymesterone (table 2).

Table 3 shows clinical characteristics of patients including factors with possible prognostic implications for the response to endocrine therapy (7). Twenty-five percent of the patients in the tamoxifen group had estrogen receptor (ER)-negative primary tumors as compared to 15% among the controls. In the total study cohort, no significant difference was observed in the distribution of metastatic site between the tamoxifen and the control patients (data not shown), but among the selected patients, there was an imbalance between the groups in this respect. Brain or liver was the dominant site in 21% and 4% in the tamoxifen and control groups, respectively. No apparent differences were observed in regard to the other recorded factors. However, the mentioned differences could introduce a bias in the overall comparison of response to treatment. Analyses were therefore also made by ER status excluding the seven patients with brain or liver metastases.

Response to treatment, duration of response, and time to treatment failure were established using criteria

TABLE 1.—Total No. of patients with disease recurrence during follow-up and reasons for exclusion from the current study by allocated adjuvant therapy

| | Adjuvant tamoxifen ($n$ = 574) | Control group ($n$ = 585) |
|---|---|---|
| ***Disease-free interval*** | | |
| Disease recurrence within 2 yrs | 63 | 88 |
| Disease recurrence after 2 yrs | 59 | 68 |
| Total | 122 | 156 |
| ***Reasons for exclusion*** | | |
| Unevaluable disease according to UICC/NCI criteria | 16 | 23 |
| Salvage surgery or radiotherapy | 7 | 8 |
| Salvage chemotherapy | 8 | 11 |
| Included in current study (ie, salvage endocrine therapy and evaluable disease) | 28 | 26 |

TABLE 2.—Response to endocrine salvage treatment by previously allocated adjuvant therapy

| Salvage treatment and response | No. of patients (%) with— Adjuvant tamoxifen | No endocrine adjuvant treatment |
|---|---|---|
| Tamoxifen | | |
| Objective response | 4 (16) | 11 (55) |
| Complete | 1 | 1 |
| Partial | 3 | 10 |
| Stable disease | 3 (12) | 4 (20) |
| Disease progression | 18 (72) | 5 (25) |
| Subtotal | 25 (100) | 20 (100) |
| Fluoxymesterone or tamoxifen and fluoxymesterone | | |
| Objective response | 0 | 3 |
| Complete | 0 | 1 |
| Partial | 0 | 2 |
| Stable disease | 0 | 3 |
| Disease progression | 3 | 0 |
| Subtotal | 3 | 6 |
| All patients | | |
| Objective response | 4 (14) | 14 (54) |
| Complete | 1 | 2 |
| Partial | 3 | 12 |
| Stable disease | 3 (11) | 7 (27) |
| Disease progression | 21 (75) | 5 (19) |
| Total | 28 (100) | 26 (100) |

TABLE 3.—Characteristics*

| Characteristic | Adjuvant tamoxifen ($n$ = 28) | No endocrine adjuvant treatment ($n$ = 26) |
|---|---|---|
| Age (yrs) | | |
| Median | 60 | 60 |
| Range | 50–70 | 51–69 |
| Previous adjuvant therapy | | |
| Chemotherapy | 12 (43) | 11 (42) |
| Radiotherapy | 9 (32) | 5 (19) |
| ER-positive treatment† | 16 (57) | 18 (69) |
| ER-negative treatment† | 7 (25) | 4 (15) |
| ER not measured | 5 (18) | 4 (15) |
| Dominant metastatic site | | |
| Soft tissue | 5 (18) | 4 (15) |
| Bone or lung | 17 (61) | 21 (81) |
| Brain or liver | 6 (21) | 1 (4) |
| ≥ 2 involved metastatic sites | 13 (46) | 12 (46) |
| Recurrence-free interval (mos) | | |
| Median | 49 | 40 |
| Range | 29–80 | 25–81 |

*Unless otherwise specified, values = No. of patients (%).

†Assay method was isoelectric focusing on polyacrylamide gel (8,9). An ER content of ≥ 0.10 fmol/μg of DNA was considered positive.

suggested by the International Union Against Cancer (UICC) and National Cancer Institute (NCI) as summarized by Hayward et al (10,11). The minimum duration of treatment before response evaluation was 1 month. The figures for objective response rate refer to the best response achieved irrespective of the time to that response. The random variation of the estimated proportions of objective responses was assessed using a normal approximation of the binominal distribution (12). The statistical significance of differences in response rate was assessed using the chi-square test. Median values for duration of response and time to progression were based on life-table estimates. Differences in time to disease progression were analyzed with the log-rank method (13). Due to the small number of responders in the tamoxifen group, differences in median duration of response were not analyzed for statistical significance.

## RESULTS

Four of the 28 patients in the tamoxifen group (14% ± 7% SE) had an objective response (complete + partial) to the selected endocrine treatments as compared to 14 of the 26 patients in the control group (54% ± 9% SE) (table 2). This difference was statistically significant ($P < 0.01$). The median duration of response was 7 months in the tamoxifen group as compared to 13 months in the control group. The median time to disease progression was significantly shorter in the tamoxifen group (4 mos) than in the control group (15 mos) ($P < 0.05$). The difference in response was similar when the analysis was restricted only to those patients who received tamoxifen as single-drug salvage treatment (table 2).

As indicated in table 3, patients with unfavorable characteristics in regard to dominant metastatic site and ER status were more frequent in the tamoxifen group. An analysis of response was therefore made by ER status excluding the seven patients with liver or brain as their dominant metastatic site (table 4). The objective response rate among those patients with ER-positive tumors was significantly lower in the tamoxifen group (23% ± 12% SE) as compared to the control group (67% ± 11% SE) ($P < 0.05$). The median time to disease progression in the tamoxifen group was 4 months as compared to 22 months in the control group ($P < 0.05$). The number of ER-negative patients was too small to permit statistical analysis.

TABLE 4.—Relationship between response to endocrine salvage treatment and previous adjuvant therapy by ER status of primary tumor*

| ER status and response | No. of patients (%) with— Adjuvant tamoxifen | No endocrine adjuvant treatment |
|---|---|---|
| ER-positive | | |
| Objective response | 3 (23) | 12 (67) |
| Complete | 1 | 2 |
| Partial | 2 | 10 |
| Stable disease | 2 (15) | 5 (28) |
| Disease progression | 8 (62) | 1 (6) |
| Subtotal | 13 (100) | 18 (100) |
| ER-negative | | |
| Objective response | 0 | 1 |
| Complete | 0 | 0 |
| Partial | 0 | 1 |
| Stable disease | 0 | 0 |
| Disease progression | 5 | 3 |
| Subtotal | 5 | 4 |
| ER not measured | | |
| Objective response | 1 | 0 |
| Complete | 0 | 0 |
| Partial | 1 | 0 |
| Stable disease | 1 | 2 |
| Disease progression | 2 | 1 |
| Subtotal | 4 | 3 |
| All patients | | |
| Objective response | 4 (18) | 13 (52) |
| Complete | 1 | 2 |
| Partial | 3 | 11 |
| Stable disease | 3 (14) | 7 (28) |
| Disease progression | 15 (68) | 5 (20) |
| Total | 22 (100) | 25 (100) |

*Patients with liver or brain metastasis are excluded.

## DISCUSSION

Breast cancer relapses are extremely variable (7). In adjuvant therapy protocols, it is therefore difficult to define a specific salvage treatment which should be given to all patients with disease recurrence. In most studies, the relapse treatment is instead decided individually for each patient by the responsible clinician (1–6). Consequently, the choice of primary treatment is influenced by a number of factors including previous adjuvant therapy, disease-free interval, and type of recurrence. The choice of second-line therapy is also influenced by the response to the first treatment. These circumstances make valid comparisons of treatment effects difficult. To circumvent some of these problems and to obtain patients with comparable disease characteristics, we selected only those patients whose disease recurred after 2 years, who had evaluable disease, and who received endocrine treatment as their first and only pharmacological relapse treatment.

These restrictions implied that only about 43% of all patients with a disease-free interval > 2 years were included in the study. The results should therefore be judged cautiously. The higher frequency of ER-negative patients and patients with visceral disease in the group who had received adjuvant tamoxifen may have accounted for some of the observed differences in outcome. However, analysis by ER level after exclusion of patients with visceral disease did not appreciably alter the results. Thus, we could not attribute the observed differences in treatment results to imbalance between the groups in regard to prognostic factors.

The study showed that it is possible to find responders to salvage endocrine treatment among patients previously treated with tamoxifen as an adjunct to primary

surgery. However, judging from earlier reports on patients without previous endocrine treatment, the rate of objective response to salvage tamoxifen in the group who had received adjuvant tamoxifen was lower and the median duration of response shorter than expected (14). Furthermore, the rate of objective response among patients from the endocrinologically untreated control group was significantly higher and their median time to disease progression significantly longer than among those from the tamoxifen group.

Previously unpublished results from the Stockholm adjuvant trial, from which the current patients were obtained, indicate a poorer survival outlook after recurrence among patients from the tamoxifen group (fig 1). The current results indicate that a decreased response to salvage tamoxifen may have contributed to this finding.

Most patients who develop recurrent disease will ultimately die from their breast cancer. Therefore, the extent to which adjuvant therapy prevents relapses will probably be more important for the long-term effects of the treatment, rather than effects on survival after relapse. The results of the randomized trial, from which the current patients were selected, have suggested a reduced number of recurrences among patients in the tamoxifen group (4). The reduced survival after relapse does therefore not contradict that tamoxifen eventually may improve long-term survival. Our findings do suggest, however, that a prolongation of the recurrence-free interval in the tamoxifen group may be modified by differences in survival after relapse and hence with time may not be translated into a corresponding increase in overall survival.

Some of our findings agree with results reported by Bonadonna et al (15) concerning the effect of salvage treatment after adjuvant CMF chemotherapy. In their study, a decreased response to endocrine therapy was observed among those who had been treated with CMF. Contrary to our results, however, there seemed to be no decrease in survival after relapses related to adjuvant therapy.

The duration of treatment in most controlled trials of adjuvant tamoxifen has been 1 or 2 years. We observed that responders to tamoxifen could be found among patients relapsing after 2 years of adjuvant therapy. These patients could perhaps have benefited from a more prolonged course of adjuvant tamoxifen. A randomized trial of 2 versus 5 years of adjuvant tamoxifen is therefore currently being undertaken in the Stockholm area.

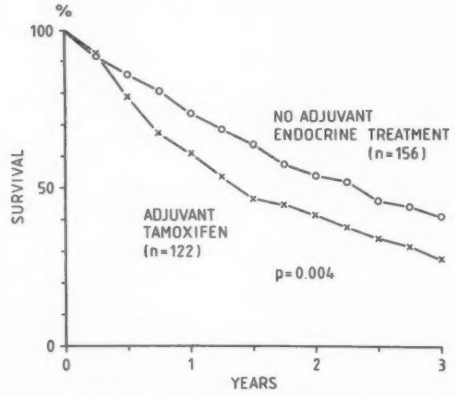


FIGURE 1.—Survival of 278 postmenopausal breast cancer patients after diagnosis of disease recurrence by previously allocated adjuvant endocrine therapy. Patients were originally included in a randomized trial of early breast cancer comparing adjuvant tamoxifen after primary surgery to no adjuvant endocrine therapy (*see* Materials and Methods section). The Nos. within parentheses indicate the total No. of patients with recurrent disease in each group at a medium follow-up of about 5 yrs. The *P* value (log-rank test) indicated a significantly poorer survival outlook after relapse for patients from the tamoxifen group as compared to the control patients.

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# High-Dose Iv Thiotepa and Cryopreserved Autologous Bone Marrow Transplantation for Therapy of Refractory Cancer[1,2]

Hillard M. Lazarus,[3,*] Michael D. Reed,[3] Thomas R. Spitzer,[3] M. Salah Rabaa,[4] and Jeffrey L. Blumer[3,5]

**Twenty-five patients with malignancies resistant to conventional chemoradiation therapy or for which no effective therapy is known were treated with escalating doses of thiotepa (135-1215 mg/m² iv over 3 days) followed by reinfusion of previously cryopreserved autologous bone marrow. The hematological and nonhematological toxic effects, therapeutic effects, and pharmacokinetics of this regimen were evaluated. Granulocyte (> 500/μl) and platelet (> 20,000/μl) count recovery occurred at a median of 16 (range, 11-38) and 18.5 (range, 11-40) days after marrow reinfusion, respectively. Six patients experienced severe infection, four of which were fatal. One patient died due to intracranial hemorrhage. Toxicity to the gastrointestinal and central nervous systems was dose-limiting, and the maximum tolerated dose of thiotepa was 1005 mg/m². Objective tumor regression occurred in six of the patients. Serum thiotepa concentration peaked immediately after infusion and declined rapidly in a biphasic manner. No relationship between thiotepa serum concentration or pharmacokinetic parameter estimates was observed for tumor response or toxicity. High-dose thiotepa and autologous bone marrow transplantation may represent an alternative therapeutic modality for patients with advanced cancer. [Cancer Treat Rep 71:689-695, 1987]**

Thiotepa, an ethylene amide developed by Lederle Laboratories in 1952, possesses mechlorethamine-like alkylating activity and has been used clinically for > 30 years (1,2). This agent, administered via a variety of routes including intratumor, oral, iv, im, intra-arterial, intrathecal, and intravesical, has been tested in vivo in many human tumors (3,4). Mild to moderate antitumor activity was observed in several solid tumors and hematologic malignancies (4-19). In particular, many of these early studies indicated that thiotepa is moderately effective therapy as a single agent administered iv in breast cancer (~ 30%), Hodgkin's disease (~ 55%), non-Hodgkin's lymphoma (~ 29%), chronic lymphocytic leukemia (~ 30%), lung cancer (~ 26%), ovarian cancer (~ 59%), chronic myelogenous leukemia (~ 60%), and malignant melanoma (~ 16%). Thiotepa is less commonly used than other alkylating agents, but finds its greatest utility in the treatment of malignant effusions, breast cancer, and intravesical therapy for bladder carcinoma.

Many alkylating agents exhibit a dose-response relationship in their anticancer activity. Thiotepa is a polyfunctional alkylating agent which could have similar efficacy in the clinical setting. We designed a phase I study employing high-dose thiotepa and reinfusion of cryopreserved autologous bone marrow for patients with refractory malignancies. We evaluated the toxicity and antitumor potential of this regimen, and determined the pharmacokinetics of thiotepa in high dose.

## MATERIALS AND METHODS

### Patient Characteristics

Twenty-five patients were studied. All patients had malignancies resistant to conventional chemoradiation therapy or for which no effective therapy is known. Nine patients had malignant melanoma, two had adenocarcinoma of unknown primary site, three had non-small cell carcinoma of lung, three had colon cancer,

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two had ovarian carcinoma, two had breast cancer, two had carcinoma of kidney, and one each had Hodgkin's disease and neuroblastoma. The median age for all patients was 52 years (range, 18–70). Fourteen of these patients were men and 11 were women. No patient had evidence of tumorous involvement of the bone marrow or had received thiotepa previously. All patients had undergone a bone marrow harvest and cryopreservation procedure at least 4 weeks from the completion of previous cytotoxic therapy. The marrow was frozen using previously described techniques and was stored in the liquid phase of a liquid nitrogen refrigerator until used (20). This protocol was approved by the Institutional Review Board for Human Subject Investigation of the University Hospitals of Cleveland, and all patients gave written informed consent.

### Evaluation and Patient Management

At the start of therapy, patient evaluation included a complete history, physical examination, chest x-ray, tumor measurement studies, complete peripheral blood cell count, and serum tests of liver and renal function. The latter three tests were obtained at least two to three times weekly during the study period and every 4 weeks after transplantation. Tumor measurement studies were performed 30 days after transplant and repeated at least every other month thereafter.

Patients were treated in single hospital rooms without specialized isolation procedures. Central venous catheters, parenteral alimentation, broad-spectrum antibiotics, and irradiated (3000 rads) blood component support were used as indicated clinically.

### Definitions

Time intervals were calculated from Day 0, the day of marrow transplantation. Complete response was defined as the disappearance of all signs and symptoms referable to tumor; partial response was defined as a $> 50\%$ reduction in cross-sectional area, without the appearance of new lesions; no response was defined as a $< 50\%$ response or stability of tumor size; tumor progression was defined as enlargement of lesions; and early death was defined as death within 30 days of marrow reinfusion. Eastern Cooperative Oncology Group (ECOG) toxicity criteria were used to grade organ system damage, except for hematologic dysfunction (21).

### Bone Marrow Infusion

Frozen bone marrow was transported to the bedside in a liquid nitrogen refrigerator for reinfusion 3 days after the final dose of thiotepa. The marrow was thawed in a 37°C water bath and directly injected iv through a central venous catheter over 5 minutes without the use of blood filters.

### Drug Administration and Sample Collection

Serum for pharmacokinetic analysis was obtained on three patients at each dose level except 1215 mg/m$^2$, where only a single patient was treated. Blood samples (5 ml) were drawn from an indwelling venous cannula before and at 2, 4, 6, 8, 10, 15, 20, 25, 30, 45, 60, 90, 120, 240, 480, and 1440 minutes after the iv bolus injection. Serum was collected after centrifugation ($1000 \times g$ for 10 minutes) at 4°C and stored at −70°C until assayed.

Instrumentation consisted of a Varian Instruments Model 5020 high-pressure liquid chromatograph (Varian Instruments, Palo Alto, CA)-fitted Rheodyne column-switching valve with a 100-$\mu$l sample loop. Absorbance of the column effluent at 210 nm was monitored on a Varian UV-100 detector interfaced with a Varian Vista CDS 401 Chromatography Data System for peak area analysis.

Thiotepa used for standardization was provided by J. G. Hackell, MD, Lederle Laboratories, Pearl River, NY. Samples for analysis were prepared in $13 \times 100$-mm glass screw-cap tubes to which 0.5 ml of serum, 1.0 ml of double-distilled water, 0.08 ml of hexamethylmelamine (internal standard; provided by L. H. Kedda, National Institutes of Health, Bethesda, MD), and 4 ml of high-performance liquid chromatography (HPLC)-grade ethyl ether (Fisher Scientific, Pittsburgh, PA) were added. The mouth of the tube was covered and the tube tightly capped. Samples were vigorously mixed and centrifuged at $100 \times g$ at room temperature for 1 minute, and 130-$\mu$l aliquots were injected onto an MCH-10 $C_{18}$ reverse-phase column (30 cm $\times$ 4 mm). The column was preceded by a 4-cm $\times$ 4-mm guard column filled with a Vydac 40-$\mu$m pellicular reverse-phase packing and was maintained at 30°C with a column heater block. A mobile phase consisting of 75% 0.06 M potassium phosphate, pH 3.0, and 25% acetonitrile at a flow rate of 1 ml/minute was used for elution. Under these conditions thiotepa eluted at 17 minutes. The analysis was linear from 0.2 (the lower limit of detection) to 50 $\mu$g/ml. The within-day coefficient of variation at 10 $\mu$g/ml ($n = 10$) was 5.9% and the between-day coefficient of variation at 10 $\mu$g/ml ($n = 14$) was 4%.

### Treatment

Thiotepa (Lederle Laboratories, Wayne, NJ) was dissolved in sterile water to a final concentration of 5 mg/ml. The first 16 patients received thiotepa by a rapid iv injection (over 1 minute) daily for 3 consecutive days as part of the pharmacokinetic study. The next nine patients were not studied for drug kinetics and received thiotepa as a 2-hour infusion daily for 3 consecutive days. With the exception of iv dexamethasone for prevention of nausea, no other drugs were given during dosing or during the blood-sampling period. The autologous bone marrow was infused 3 days after the last dose of thiotepa.

### Dose Escalation

Thiotepa (135 mg/m$^2$) was chosen as the initial dose since doses as high as 60 mg/m$^2$ were used without marrow reinfusion (22). One-third of the total dose was

administered each day for 3 consecutive days. The total dose in milligrams per meter-squared therefore is the sum of the three consecutive daily doses. Subsequent dose escalations (modified from the methods of Fibonacci) were 270, 405, 810, 1005, and 1215 mg/m$^2$. With the exception of the highest dose level, groups of at least three patients were entered at each dose level. Patients were then observed for signs of toxicity for a minimum of at least 4 weeks.

### Pharmacokinetic Analysis

Both model-dependent and -independent techniques were used to describe the biodisposition of thiotepa (23). Serum thiotepa concentrations for each patient were plotted against time on a semilogarithmic scale. The area-under-the-serum-concentration-time curve (AUC) was obtained by using the linear trapezoidal rule up to the final measured serum concentration and extrapolated to infinity. The elimination half-life was determined by using the postdistributive terminal portion of the serum concentration-time curve. Body clearance (Cl) was determined by the formula dose/$AUC_0^\infty$. The apparent steady-state volume of distribution ($Vd_{ss}$) was determined by the formula:

$$Vd_{ss} = [\text{dose}(AUMC)/AUC^2] - (\text{dose} \cdot T/AUC^2),$$

where AUMC is the area-under-the-moment curve and T is the infusion duration. In addition, thiotepa serum concentration-time curves were assessed using a computer program (AUTOAN) for goodness of fit to a two-compartment model. In applicable patients, serum concentration-time data were weighted to the best fit ($1/y^2$) and analyzed using a computer program (NONLIN) and standard two-compartment methods (24). Statistical analysis was performed using the paired Student's t test, linear regression, and multivariant analysis.

## RESULTS

Three patients were treated with thiotepa at a dose of 135 mg/m$^2$, four at 270 mg/m$^2$, four at 540 mg/m$^2$, five at 810 mg/m$^2$, eight at 1005 mg/m$^2$, and one at 1215 mg/m$^2$. The median dose of bone marrow reinfusion was $1.4 \times 10^8$ nucleated marrow cells/kg (range, 0.9–2.0).

The organs that were affected most by the thiotepa were bone marrow, gastrointestinal tract, and CNS. Wbc and platelet counts fell rapidly after thiotepa infusion. Recovery of peripheral blood cell counts usually occurred within several weeks after marrow reinfusion. Of the 25 patients entered in the study, two died of tumor progression within 30 days of initiating therapy, one each died of bleeding and hepatic failure, and four died of infection. Seventeen patients survived at least 30 days after marrow transplant to be considered evaluable for recovery of marrow function. The peripheral polymorphonuclear neutrophil count was $> 500$ $\mu$l for a median of 16 days (range, 11–38) after marrow reinfusion. Correspondingly, platelet count was $> 20{,}000/\mu$l without the aid of transfusion for a median of 18.5 days (range, 11–40) after transplantation. One patient never recovered a platelet count $> 20{,}000/\mu$l despite a prompt recovery in peripheral polymorphonuclear neutrophil count ($> 500/\mu$l at Day 15 and $> 1500/\mu$l at Day 20). This patient developed clinical evidence of low-grade disseminated intravascular coagulation and tumor progression during therapy. He refused a diagnostic bone marrow aspirate and biopsy. Recovery of marrow function was not influenced by factors such as number of courses of previous chemotherapy before marrow cryopreservation, total thiotepa dose administered, number of nucleated marrow cells infused per kilogram, and interval from cryopreservation to infusion of the marrow.

Severe toxicity at thiotepa dose levels of 810–1215 mg/m$^2$ is shown in table 1. None of the 11 patients who received $\leq$ 540 mg/m$^2$ developed ECOG grade 2 or greater organ dysfunction that could be attributed to thiotepa, although one patient had bacteremia that was successfully treated. Five patients treated at the 810–1215-mg/m$^2$ dose level experienced severe infections while neutropenic, four of which were fatal (table 1). One patient with malignant melanoma metastatic to the brain developed a spontaneous intracranial hemorrhage at a time when the platelet count was $55{,}000/\mu$l and she died as a result of this occurrence. This phenomenon may have been due to bleeding into the melanoma brain lesions, which has been observed previously (25). A second patient with a platelet count of $19{,}000/\mu$l developed a subdural hematoma, which was surgically drained, and the patient was then discharged.

Toxicity to the gastrointestinal system (mucositis) and CNS appeared related to the thiotepa dose administered and was dose-limiting (table 1). One of five patients treated at 810 mg/m$^2$ developed grade 3 stomatitis,

TABLE 1.—Severe toxicity at thiotepa dose levels of 810–1215 mg/m$^2$*

| Thiotepa dose (mg/m$^2$) | No. of patients | Toxic effects (No. of deaths) | | | | |
|---|---|---|---|---|---|---|
| | | Infection | Hemorrhage | Mucositis | Hepatic | CNS |
| 810 | 5 | 2 (1) | 2 (1) | 1 | — | — |
| 1005 | 8 | 2 (2) | — | 3 | 1 (1) | 3 (1) |
| 1215 | 1 | 1 (1) | — | 1 | — | — |
| Total | 14 | 5 (4) | 2 (1) | 5 | 1 (1) | 3 (1) |

*Defined as $\geq$ ECOG grade 3 toxicity (ref 21).

esophagitis, and mucositis of a nephrostomy site. She died 18 days after transplantation due to *Escherichia coli* septicemia. In the group of nine patients who received 1005–1215 mg/m$^2$, severe mucositis, CNS dysfunction, or hepatic failure occurred in six patients. Two patients developed grade 3 gastrointestinal toxicity only (diarrhea, stomatitis, and esophagitis), while one patient each experienced isolated grade 3 CNS dysfunction (somnolence) and fatal hepatic failure. Two patients experienced both gastrointestinal (or mucosal) and CNS toxicity; one of these patients died as a result of the brain injury (seizures and later coma), while the other patient died of bacteremia. Other toxic effects which could be attributed to high-dose thiotepa occurred in one patient in the group treated at the 810-mg/m$^2$ level, who developed an unusual bronzing of the skin.

Of 19 patients evaluable for analysis for antitumor response, no patient achieved complete response and six achieved partial response. Two malignant melanoma patients with visceral metastases achieved partial response lasting 3 and 4 months. The other partial responses occurred in individual patients with non-small cell lung cancer (7 months' duration), breast cancer (4 months' duration), carcinoma of the kidney (3 months' duration), and refractory Hodgkin's disease (3 months' duration). Four patients experienced tumor progression, two of which were fatal, within 30 days after transplant.

### Pharmacokinetic Analysis

The bioexponential thiotepa serum concentration-time curve after the first day's drug dose for the six different dosage groups is shown in figure 1. Group 1 included those patients who received thiotepa at 45 mg/m$^2$/day by iv bolus for 3 days (total, 135 mg/m$^2$); Group 2 patients received 90 mg/m$^2$/day (total, 270); Group 3 patients received 180 mg/m$^2$/day (total, 540); Group 4 patients received 270 mg/m$^2$/day (total, 810); Group 5 patients received 335 mg/m$^2$/day (total, 1005); and Group 6 was the single patient who was treated with thiotepa at 405 mg/m$^2$/day by iv bolus for 3 days (total, 1215 mg/m$^2$). The highest thiotepa serum concentration observed occurred 2 minutes after bolus drug administration. Two-minute peak thiotepa serum concentrations averaged 4.6, 6.7, 12.1, 16.7, 38.9 ($n$ = three patients for each of the five groups), and 25.7 μg/ml ($n$ = one patient) for the six different dose groups, respectively. These serum concentrations correlated directly with the bolus dose administered ($r = 0.74$ on Day 1; $r = 0.89$ on Day 3; $P < 0.001$). Thiotepa concentrations determined at the 8-hour sampling time were measurable in all but one patient (Group 2) on study Day 1 and again in all patients on study Day 3. Eight-hour thiotepa concentrations after the first dose ranged from 0.2 to 2.7 μg/ml. At higher doses, the 8-hour concentration was 50% of the peak concentration at the lowest dose. For both study Days 1 and 3, 24 hours after drug administration residual serum thiotepa concentration could not be observed above the detection limit.

The results of model-independent and two-compartment pharmacokinetic analysis for the first day of therapy are shown in table 2. Statistical evaluation of half-life, $Vd_{ss}$, Cl, and AUC by the paired Student's t test revealed no differences between model-independent and two-compartment analysis among the various dosage groups or between study Days 1 and 3. The thiotepa half-life and body Cl approximated 2.5 hours and 403.1 ml/minute/1.73 m$^2$, respectively. Linear regression analysis of thiotepa dose (mg/m$^2$) versus AUC demonstrated

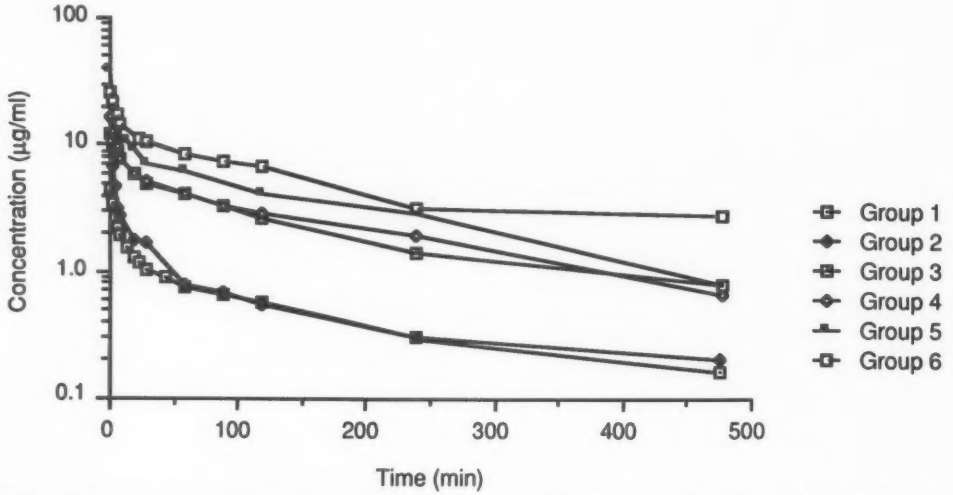


FIGURE 1.—Thiotepa serum concentration-time curve by patient dose group.-□-= Group 1 ($n$ = 3);-◆-= Group 2 ($n$ = 3);-⊞-= Group 3 ($n$ = 3);-◇-= Group 4 ($n$ = 3);-▬-= Group 5 ($n$ = 3); and-□-= Group 6 ($n$ = 1).

TABLE 2.—Comparative first-dose thiotepa pharmacokinetic parameter analysis in 16 patients

| Parameter | Noncompartment | 2-compartment |
|---|---|---|
| Half-life (hrs) | 2.5 ± 1.3 SD | 2.6 ± 1.5 SD |
| $Vd_{ss}$ (L/kg) | 1.1 ± 0.4 SD | 1.0 ± 0.3 SD |
| Cl (ml/min/1.73 $m^2$) | 403.1 ± 234 SD | 465.7 ± 321 SD |
| AUC (μg/ml·hr) | 20.6 ± 17 SD | 19.7 ± 18 SD |
| α-Half-life (hr) | — | 0.11 ± 0.11 SD |
| $V_c$ (L/kg) | — | 0.32 ± 0.16 SD |

a direct linear relationship on both study days (Day 1, $r = 0.82$, $P < 0.001$; Day 3, $r = 0.92$, $P < 0.001$). The half-life of distribution (α-half-life) averaged 0.1 hours and the volume of the central compartment $V_c$ averaged ⅛ of the overall $Vd_{ss}$ (0.32 L/kg). No relationship was identified between thiotepa pharmacokinetic parameter estimates and patient body weight (kilogram), surface area ($m^2$), tumor response, or overall patient outcome.

## DISCUSSION

Substantial experimental and clinical evidence supports a steep dose-response relationship for many cytotoxic drugs, particularly ionizing radiation and alkylating agents, when used to treat sensitive tumors such as hematologic malignancies (12,26–34). In some diseases refractory to conventional forms of therapy, high-dose chemotherapy (with or without radiation therapy) and marrow infusion are associated with cure in a variety of malignant diseases (35,36). In the present investigation we studied an agent that has been utilized infrequently in humans in this fashion (37). Early studies suggested that a moderate dose-response effect is identifiable with thiotepa (12). In a group of 25 patients with advanced malignancies we demonstrated that thiotepa could be administered in significantly increased doses with the use of cryopreserved autologous bone marrow reinfusion. We chose to administer thiotepa over 3 days as we and other investigators have done previously with other high-dose alkylating-agent regimens (38–40).

All patients experienced severe neutropenia and thrombocytopenia. Thiotepa dose did not appear to influence the rate or degree of marrow suppression. The six evaluable patients treated at the initial two levels (135 and 270 mg/$m^2$), however, developed minimal extramedullary toxicity. At the higher doses, four patients died as a result of severe infection, and thrombocytopenia and intracranial hemorrhage caused the death of a patient who had malignant melanoma brain metastases.

Mucositis and CNS toxicity were dose-limiting. Five of 14 patients who received ≥ 810 mg/$m^2$ experienced moderate to severe diarrhea, stomatitis, esophagitis, or other mucosal injury. These toxic effects on the gastrointestinal tract are similar to the phase I animal data in dogs and mice in which gastrointestinal injury was dose-limiting (41,42).

Damage to the CNS occurred in three patients, all treated at the 1005-mg/$m^2$ dose level. These findings suggest that thiotepa, which is very lipid-soluble and achieves excellent CNS penetration, may be neurotoxic at high doses (22,43–47). Thiotepa and its metabolites were not measured in cerebrospinal fluid, and postmortem material was not available on these patients to corroborate this speculation.

Another group has recently reported on the use of high-dose thiotepa and autologous marrow transplantation, administering doses > 1125 mg/$m^2$ (37). They did not comment on CNS injury, but did note the common occurrence of gastrointestinal toxicity. This group also described a frequently occurring diffuse skin rash, which we observed in only one patient.

Plasma pharmacokinetics of thiotepa have been poorly characterized in the past due to the lack of suitable methods for analysis of drug and metabolites. These early studies utilized cumbersome, nonspecific, and insensitive techniques such as radiolabeled drug (48–52), paper chromatography (49,50), fluorometric assay (53), or spectrophotometric assay (54–56). More recently, improved techniques have been utilized and include proton magnetic resonance spectrometry (57) and gas-liquid chromatography (45,58). Although more accurate, these later methods have not routinely been employed in patients receiving high doses of drug. Interspecies variation of pharmacokinetic data varied considerably and limits the use of extrapolation to humans (46,49, 51,53). In the present study using a specific HPLC method, peak thiotepa serum concentrations occurred immediately after drug infusion with an increase coinciding with each higher dosage level. The biodisposition characteristics of the drug did not differ from the first to third dose of thiotepa, unlike many anticancer drugs that enhance or retard their own metabolism or excretion (59). The pharmacokinetics appear to be linear, and suggest that utilization of drug does not exceed its clearance.

High-dose thiotepa behaved pharmacokinetically in a manner similar to low-dose, and the data fit a two-compartmental model with most of the parent drug disappearing by 8 hours (58,60). Recognized active metabolites such as tepa (triethylenephosphoramide) and other unidentified metabolites which also possess alkylating-agent activity were not assayed in this study (58,60). The contribution made by such metabolites to toxicity and efficacy in our study cannot be ascertained, and additional studies are required since the actual pharmacokinetic data of thiotepa alone may not completely correlate with observed in vivo activity.

Tumor responses were noted in six patients in this small phase I series. One relatively durable partial response (which lasted 7 months in a patient with progressive non-small cell lung cancer after radiation therapy) was noted in a patient who received 135 mg/$m^2$ of drug, a relatively low dose. Antitumor responses of brief duration were seen in other malignancies; the increased toxicity at higher doses of thiotepa was not accompanied by more frequent or durable responses in our group of cancer patients.

In summary, we believe the maximum tolerable dose of high-dose thiotepa to be 1005 mg/m$^2$ when administered in conjunction with autologous bone marrow transplantation. Toxicity to the marrow, gastrointestinal tract (mucositis), and CNS are the major side effects (table 1), especially in a series of patients that tend to be a bit older than those reported in most autologous bone marrow transplant trials. Thiotepa pharmacokinetics in high dose are similar to those at lower doses; these are an important set of data when planning marrow reinfusion timing and scheduling with other agents in high-dose combination chemotherapy and marrow reinfusion. Single-agent high-dose drug studies are important to identify agents that can be combined in high-dose regimens based on shared activity against particular tumors and different dose-limiting toxic effects (20). The role of thiotepa in high-dose combination alkylating-agent regimens remains to be defined (61).

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# In Vitro Chemosensitivity of Human Lung Cancer Cell Lines[1]

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**Human lung cancer cell lines, established from patients with both small cell cancer (SCLC) and non-small cell cancer (non-SCLC; squamous cell, large cell, anaplastic, and adenocarcinoma), were tested for their in vitro chemosensitivity to a panel of drugs. Drug sensitivity was assayed by either soft agar clonogenicity or a novel dye-exclusion assay. Eleven non-SCLC lines (eight continuous, three recently cultured) and five SCLC lines (all continuous) were tested. Four of eight continuous non-SCLC lines cloned sufficiently to permit limited in vitro drug testing, as did two of the five SCLC lines. All 16 cell lines could be tested for multiple drugs using the dye-exclusion assay. Drug concentrations for the nonclonogenic assay more closely approximated the area under the concentration-time curve for a given concentration of each agent. There was considerable variation in the relative sensitivity of the cell lines and the patterns of individual drug sensitivity. The majority of non-SCLC cell lines were refractory to most drugs. Cell lines derived from two previously treated SCLC patients and from three untreated SCLC patients showed greater sensitivity. Concurrent clonogenic and dye-exclusion assays showed similar drug rankings but different absolute values for percent survival. The nonclonogenic dye-exclusion assay is more rapid than the soft agar clonogenic assay (4 days vs. 2-3 weeks), could be performed on all cell lines tested, and appears to reflect the clinical diversity of human lung cancer. [Cancer Treat Rep 71:697-704, 1987]**

The treatment of advanced human lung cancer is a clinically frustrating problem that requires careful balancing of risks and potential benefits. Although initial responses to therapy for small cell lung cancer (SCLC) are dramatic, the overwhelming majority of patients will relapse within 1 year with clinically refractory disease (1). Advanced non-SCLC is even more discouraging, with less than half of all patients responding to therapy and no clear-cut impact on survival or quality of life (2). Most patients will receive potentially toxic therapy that has no impact on their cancer. The need for a reliable, in vitro assay to predict clinical responsiveness is pressing.

Prediction of in vivo drug sensitivity on the basis of in vitro results has been a goal of researchers for > 30 years. In recent years the soft agar clonogenic assay, popularized by Salmon et al (3), has become the standard assay for in vitro drug selection. Although there are conceptual reasons that the clonogenic assay may not be optimal for prediction of clinical efficacy (4), its major limitations are technical (reviewed in ref 4). The need to obtain single-cell suspensions is the most serious drawback, leading to artifacts caused by the methods of cell dissociation (5) and to difficulty in interpreting the results due to the presence of "clumps" rather than "colonies" (6,7). In our experience, the mechanical or enzymatic disaggregation of SCLC specimens or cell lines to a strict single-cell suspension results in the death of the overwhelming majority of tumor cells. Even when the technical problems of the assay are minimized, the ability to study individual patient samples is limited by the poor colony-forming efficiency of lung cancer (8-10). SCLC specimens will proliferate in agarose only to the degree that limited drug testing can be done in approximately 20% of cases (11). This inability to test most specimens has been reported by a number of laboratories (4,12).

Weisenthal and his colleagues (13) have recently reported a novel dye-exclusion assay that is reported to overcome many of the theoretical and practical problems associated with dye-exclusion assays and can be performed on > 80% of clinical specimens. There are a series of conceptual advantages to measuring cell kill in both the dividing and nondividing cell populations (4),

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and the ability to interpret the results in the presence of tumor cell clusters and nonmalignant cells permits maintenance of tumor cell heterogeneity and cell-cell interactions (14).

Our understanding of the biology of human lung cancer has been greatly facilitated by the development of a large panel of both SCLC and non-SCLC cell lines (15,16). This study utilizes those cell lines to ascertain in vitro chemosensitivity using both the nonclonogenic dye-exclusion assay reported by Weisenthal and the traditional soft agar clonogenic assay.

## MATERIALS AND METHODS

### Cell Lines

All cell lines were derived from human tumor specimens as previously described (15,16). Long-term cultures were grown in RPMI-1640 medium with 10% fetal bovine serum and 1 mM glutamine (R10). All media and sera were obtained from Grand Island Biological Co, NY. Horse serum, as described by Weisenthal et al (13), was not added to the basic culture medium. The cultures were maintained at 37°C in a humid 5% $CO_2$ atmosphere. All of the continuous cell lines have been characterized by cytology, flow cytometry, isozyme analysis, nude mouse tumorigenicity and a series of neuroendocrine and epithelial markers. The short-term cultures were handled and characterized identically; they differ only in not having been continuously subcultured for $> 6$ months. All cultures were free of *Mycoplasma* contamination, as determined by repeated testing performed by Microbiological Associates, Bethesda, MD. Assays were performed using cells in logarithmic growth phase. Cell lines established in our laboratory have the prefix NCI- (eg, NCI-H69), where the H connotes derivation directly from a clinical human specimen and N connotes prior passage in nude mice (eg, NCI-N417). The prefix NCI- is dropped in the text for clarity.

### Preparation of Cell Lines for Drug Testing

Non-SCLC cell lines are characterized by substrate adherence and grow as monolayer cultures. They were detached by trypsinization, pipetted gently in the culture medium (R10) used for neutralization, washed once, and resuspended in R10. Cells were counted in a standard hemacytometer chamber using an inverted phase microscope. When cells were brought to strict single-cell suspensions for the clonogenic assay, significant cell death was shown by trypan blue testing, with classic SCLC lines suffering more damage than non-SCLC lines. Variant SCLC lines demonstrated intermediate toxicity. Following trypsinization, the cells were allowed to incubate in R10 for at least 4 hours before drug testing, to permit regeneration of cell surface components. SCLC cell lines grow as floating aggregates with the density and adherence of the aggregates related to whether the cell line is a "classic" or "variant" SCLC cell line as described elsewhere (17,18). SCLC lines were disaggregated by pipetting through a sterile Pasteur pipette. For both SCLC and non-SCLC lines, the cell suspensions were pipetted to single-cell suspensions for clonogenic testing and to small clusters (one to six cells) for the nonclonogenic, dye-exclusion assay.

### Drug Preparation and Doses

The drugs tested, their preparation, and the dosages employed for each assay are listed in table 1. 4-Hydroperoxycyclophosphamide was kindly provided by Dr. Michael Colvin, The Johns Hopkins Hospital, Baltimore, MD. Procarbazine, melphalan, and phosphoramide mustard were provided by the National Cancer Institute in powdered form. All other drugs were obtained from routine commercial sources via the clinical pharmacy. In addition, the area under the concentration:time curve (AUC) for a "standard" clinical dose of the drug was calculated using the equation AUC = dose ÷ clearance, where AUC is expressed as μg/ml/hour. Drugs were prepared to a concentration ten times the desired final concentration in phosphate-buffered saline (PBS) or sterile water, and 0.1 ml was diluted in 0.9 ml of the culture medium containing the cell suspension being tested to obtain the final concentration. Cells were exposed to drug for 1 hour, with the exception of methotrexate which was maintained as a continuous exposure. Drugs were prepared as outlined (table 1) and maintained at −70°C, with the exception of lomustine (CCNU) which was prepared fresh for each experiment. Drug solutions were sterilized by filtration through 0.22-μm pore (diameter) filters (Millipore Co, Bedford, MA). Control tubes were incubated with PBS. All tests were done in duplicate.

### Nonclonogenic Dye-Exclusion Assay

Cells were diluted to a concentration of $5 \times 10^4$ cells/assay tube and placed in 4.5-ml conical polypropylene tubes (VWR Scientific, Norwalk, CA). The polypropylene tubes were used to discourage cell attachment (13), and their conical shape facilitated aspiration of supernates prior to staining. Following the 1-hour drug exposure, 3 ml of R10 was added, the cells were centrifuged at 1200 rpm for 7 minutes, the supernate was decanted, and two further washes with 1 ml of R10 were done. The cells were then resuspended in 1.0 ml of R10 and incubated for 4 days at 37°C in a humidified 5% $CO_2$ atmosphere.

Duck erythrocytes (DRBC) were fixed and sterilized as described previously (13). Following the 4-day incubation, 0.1 ml of the DRBC suspension ($1 \times 10^6$ DRBC/ml) was added to each tube, the resulting mixture vortexed and centrifuged at 1200 rpm for 7 minutes, and the supernate carefully aspirated. From 0.1 to 0.2 ml of a 1:1 (vol:vol) mixture of 2% fast green and 1% nigrosin (19) was added, and the tubes were vortexed and then incubated at 20°C for 10 minutes. The stained cell suspension was then cytocentrifuged (Shandon Southern

TABLE 1.—Drug preparation and dosages for the clonogenic and the nonclonogenic, dye-exclusion assays

| Drug | Solvent(s)* | In vitro dose (μg/ml)† | | AUC dose (μg/ml/hr) | Clinical dose | Reference No. |
|---|---|---|---|---|---|---|
| | | Clonogenic | Dye-exclusion | | | |
| Doxorubicin | PBS | 0.06 | 1.2 | 1.98 | 60 mg/m$^2$ | 30 |
| Mitomycin | SW, PBS | 0.1 | 0.8 | 0.55 | 10 mg/m$^2$ | 31 |
| Cisplatin | SW, PBS | 0.2 | 6.6 | 4.76 | 100 mg/m$^2$ | 32 |
| Etoposide | PBS | 3 | 125 | 41.7 | 100 mg/m$^2$ | 33 |
| Vinblastine | PBS | 0.05 | 1.0 | 0.22 | 6 mg/m$^2$ | 34 |
| Vincristine | PBS | 0.01 | 0.8 | 0.36 | 1.4 mg/m$^2$ | 34 |
| Procarbazine | PBS | ND | 10 | —§ | | — |
| Methotrexate | PBS | 0.3‡ | 20‡ | 6.1 | 40 mg/m$^2$ | 35 |
| CCNU | ETOH, PBS | 0.1 | 10 | —§ | | 35 |
| Melphalan | ETOH, PBS | ND | 10 | 0.16 | 0.6 mg/kg | 36 |
| Mechlorethamine (nitrogen mustard) | SW, PBS | ND | 3.5 | —§ | | — |
| Phosphoramide mustard | PBS | ND | 10 | 19 | 10 mg/kg | 37 |
| 4-Hydroperoxycyclophosphamide | PBS | ND | 10 | 57¶ | 10 mg/kg | 37 |
| Cyclophosphamide | SW, PBS | ND | 100 | 109 | 10 mg/kg | 38 |

*First diluent listed brings powder to solution. All drugs to final concentration in PBS. SW = sterile water; ETOH = absolute ethyl alcohol.
†ND = not done.
‡Continuous exposure; all other drug exposures for 1 hour.
§No data available on drug levels of procarbazine, CCNU, and mechlorethamine.
¶Calculated as 3 × AUC for phosphoramide mustard per ref 37.

Instruments, Inc., Sewickley, PA) for 8 minutes at 1200 rpm, and the slides were air dried. Glass slides were previously washed in 70% ethanol. Counterstaining was accomplished using Harris formula hematoxylin solution (Harleco, Gibbstown, NH) to which glacial acetic acid was added to a final concentration of 2%. Details of the counterstaining procedure have been published (13) and reflect the need to stain the cells without prior fixation. In short, four brief rinses in 7.5% ethanol were followed by 30 seconds in eosin solution [1% eosin (100 ml):1% phloxine (10 ml):95% ethanol (780 ml):glacial acetic acid (4 ml)] and dehydration successively in 95% ethanol, 100% ethanol, and xylene. Slides were coverslipped with mounting balsam and scored as described below.

Scoring of the slides was accomplished by determining the ratio of live tumor cells to DRBC for each slide and comparing this ratio to that for the control (PBS-treated) slides. In practice this was done by counting the number of DRBC and live tumor cells in the same high-power fields (100×) until 200–300 DRBC were counted. If the cell content was too high to allow accurate counting of an entire field, an eyepiece grid (Whipple disc, Bausch and Lomb No. 31-16-69; VWR Scientific, Norwalk, CA) was used to establish appropriate sectors on the control slides, and these were counted on the experimental slides as well. Individual cells were counted in clumps of 2–20 cells; cells in larger clumps were not counted. Initially, a solution of 30 mM EDTA:10 mM dithiothreitol dissolved in PBS was added at the same time as the DRBC to reduce clumping as suggested by Weisenthal (13). It was, however, ineffective. Live tumor cells stained normally with hematoxylin and eosin and were readily distinguishable from the green-black coloration of the dead cells. Fast green:nigrosin was used instead of the fast green originally reported, as the green staining is not visible to color-blind observers and the combination has been shown to be equivalent (19). DRBC are immediately identifiable as nucleated elliptocytes. The use of DRBC as an internal standard that minimizes the impact of early cell lysis and continued proliferation of surviving cells is an important advance in quantifying dye-exclusion assays, as described by Weisenthal et al (13) and others (19). The percent survival for a specific concentration of an individual drug is defined as:

$$\% \text{ survival} = \frac{(\text{No. of live tumor cells} \div \text{No. of DRBC})\ \text{exp}}{(\text{No. of live tumor cells} \div \text{No. of DRBC})\ \text{control}} \times 100.$$

### Clonogenic Assay

Following the 1-hour drug exposure cells were washed twice with R10 and suspended in a mixture of R10 and agarose (0.3%; Marine Colloid Division, FMC Corp., Rockland, ME) maintained at 40°C (11). The cells in agarose were plated over a base layer of 1.5 ml of agarose (0.5%) prepared the night before in 35-mm dishes. Colonies of ≥ 50 cells were counted 14–21 days after plating. Plates were checked for clusters 1 day after plating.

### Plating Efficiency

Cells from non-SCLC lines were diluted in R10 to $10^3$–$10^5$/ml, 1 ml was placed in a 35-mm sterile tissue culture dish, and the cells were dispensed evenly by gentle rotary motion. Seven to 21 days later, the plates were decanted, washed once in PBS, and stained with crystal violet. The number of colonies per number of cells plated was calculated as the plating efficiency.

TABLE 2.—Colony-forming and plating efficiency of non-SCLC continuous cell lines

| Lung cancer cell line | Tumor diagnosis | CFE (%)* | PE (%)† |
|---|---|---|---|
| H207 | Adenocarcinoma | 3.2 | 17.3 |
| H358 | Adenocarcinoma | 0 | 11.0 |
| H460 | Large cell carcinoma | 1.8 | 5.6 |
| H23 | Adenocarcinoma | 0 | 4.0 |
| H157 | Large cell carcinoma | 0 | 2.4 |
| H125 | Adenocarcinoma | 0 | 0.8 |
| H324 | Adenocarcinoma | 0 | 3.7 |
| H520 | Squamous cell carcinoma | 0.002 | 0.01 |

*Colony-forming efficiency determined from No. of colonies/No. of cells plated in agarose.

†Plating efficiency determined from No. of colonies/No. of cells plated on a 35-mm tissue culture plate.

Colony-forming and plating efficiency for the eight continuous non-SCLC cell lines are depicted in table 2.

### Experimental Design

All of the cell lines were tested using the nonclonogenic dye-exclusion assay. Concentrations of drugs employed were those reported by Weisenthal et al (13); as demonstrated in table 1, these more closely approximate AUC doses than the routine concentrations employed in the clonogenic assay. Concentrations of drugs in the clonogenic assay were those reported by Alberts and Chen (20). All of the continuous cell lines were tested for soft agar clonogenicity, and lines that gave satisfactory growth were used for the comparisons between the assays. Comparisons were performed by aliquoting the cell suspensions and simultaneously performing the two assays. Both assays studied a hundredfold range of drug concentrations [tenfold above and below the "standard" (1×) concentration] and results are the mean of duplicate scores.

## RESULTS

### Non-SCLC Cell Lines

Eight continuous cell lines were tested against four agents clinically useful in the management of advanced non-SCLC: doxorubicin, mitomycin, cisplatin, and etoposide (table 3). None of the patients from whom these cell lines were developed had received prior therapy. Using an arbitrary standard of < 50% survival at the standard concentration (doxorubicin, 1.2; mitomycin, 0.8; cisplatin, 6.6; and etoposide, 125 μg/ml) as a measure of in vitro responsiveness, only one line was sensitive to doxorubicin (H460), mitomycin (H207), and cisplatin (H460), whereas two lines (H358 and H460) were responsive to etoposide. Five cell lines (H23, H157, H125, H324, and H520) were refractory to all four drugs tested at the 1× concentration. H460 was responsive to three of the four drugs tested. All of the cell lines tested showed marked responsiveness to a 10× concentration of etoposide.

Three recently cultured cell lines and four fresh specimens were tested against the same panel of drugs with similar results (table 3). H676A was from a patient with massive malignant pleural effusion from adenocarcinoma of the lung. He had previously received cyclophosphamide, doxorubicin, methotrexate, and procarbazine. H676B was established after the patient had a minor regression followed by progression on vinblastine plus cisplatin. Only the response to doxorubicin appeared

TABLE 3.—In vitro chemosensitivity* of non-SCLC cell lines to a panel of drugs†

| | Doxorubicin (μg/ml) | | | Mitomycin (μg/ml) | | | Cisplatin (μg/ml) | | | Etoposide (μg/ml) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 12 | 1.2 | 0.12 | 8 | 0.8 | 0.08 | 66 | 6.6 | 0.66 | 1250 | 125 | 12.5 |
| Established cell lines | | | | | | | | | | | | |
| H207 | 77 | 64 | 68 | 22 | 49 | 52 | 34 | 64 | 72 | 0 | 69 | 74 |
| H358 | 78 | 96 | 89 | 88 | 80 | 88 | 84 | 88 | 97 | 43 | 42 | 60 |
| H460 | 18 | 19 | 40 | 18 | 60 | 50 | 2 | 45 | 44 | 0 | 27 | 59 |
| H23 | 3 | 81 | 85 | 69 | > 100 | > 100 | 93 | 96 | > 100 | 0 | 70 | > 100 |
| H157 | 30 | > 100 | > 100 | > 100 | > 100 | > 100 | 66 | > 100 | > 100 | 2 | > 100 | > 100 |
| H125 | 33 | 55 | 92 | 26 | 69 | 98 | 89 | 99 | > 100 | 0 | 60 | 91 |
| H324 | > 100 | 74 | > 100 | > 100 | > 100 | > 100 | > 100 | ND | ND | 0 | 96 | 72 |
| H520 | > 100 | > 100 | > 100 | > 100 | 85 | > 100 | > 100 | > 100 | > 100 | 0 | > 100 | 98 |
| Recently cultured | | | | | | | | | | | | |
| H661 | 43 | 95 | > 100 | 39 | 99 | 59 | 30 | > 100 | > 100 | 1 | 51 | > 100 |
| H676A | 48 | 49 | 61 | 84 | 69 | 87 | 36 | 50 | 86 | 4 | 36 | > 100 |
| H676B | > 100 | > 100 | > 100 | > 100 | 99 | 95 | 40 | 76 | > 100 | 2 | 70 | 33 |
| Fresh specimens | | | | | | | | | | | | |
| H697 | 15 | 76 | > 100 | 12 | 64 | > 100 | ND | ND | 65 | 0 | 95 | > 100 |
| H709 | ND | 82 | ND | 69 | 80 | > 100 | > 100 | > 100 | > 100 | 0 | 66 | > 100 |
| H717 | 65 | > 100 | 95 | 62 | 58 | 95 | 99 | 99 | > 100 | 2 | 67 | > 100 |
| H718 | 64 | > 100 | > 100 | 89 | > 100 | > 100 | > 100 | > 100 | > 100 | 10 | 70 | > 100 |

*In vitro chemosensitivity by nonclonogenic dye-exclusion assay (13).

†Values in table are % survival. ND = not determined.

to change consistently. Four of the newly cultured (H676A and H676B) and fresh (H717 and H718) specimens were also tested against vinblastine, methotrexate, mechlorethamine, CCNU, and procarbazine. No responses were seen with the exception of the response of H676A to CCNU.

### SCLC Cell Lines

Table 4 displays the results for five continuous and one short-term SCLC cell lines. In the lines from previously untreated patients (H209, H187, H417, and H678), there were 16 instances where data were available for the standard dose of a drug; on ten of those occasions cell survival was < 50%. This does not differ from the three of seven responses seen for cell lines from previously treated patients, although the pattern is towards greater responsiveness of untreated cell lines. All lines were responsive to 10× increases in the doses of etoposide, doxorubicin, vincristine, and methotrexate, but not cisplatin.

### Comparison of Clonogenic and Dye-Exclusion Assays

Table 5 displays the rank order of drugs selected by the two assays. N417 is a variant SCLC cell line from a patient without prior therapy. The two assays demonstrate clear responsiveness to etoposide, relative refractoriness to melphalan and cisplatin, and absence of response to CCNU. The clonogenic assay did not detect responsiveness to vincristine or doxorubicin, two of the more effective agents in clinical usage. H146 is a classic SCLC cell line derived from a previously treated patient. Both tests demonstrate a relative paucity of response to all agents tested.

### In Vitro Testing of Cyclophosphamide

Because cyclophosphamide requires in vivo hepatic activation it cannot routinely be used as a test drug in in vitro situations (21). Despite attempts at in vitro activation with rat hepatic microsomes (22), most investigators have substituted either melphalan (3) or mechlorethamine (13) as "equivalent" alkylating agents, despite the knowledge that they are unlikely to be truly equivalent. We examined the response of H417 to mechlorethamine, melphalan, phosphoramide mustard, 4-hydroperoxycyclophosphamide, and cyclophosphamide itself (fig 1). Cyclophosphamide alone had no effect. Only 4-hydroperoxycyclophosphamide had a significant dose response effect.

TABLE 5.—Comparison of dye-exclusion and clonogenic assays

| Rank order (% survival)* | | | |
|---|---|---|---|
| Dye-exclusion assay | | Clonogenic assay | |
| ***Cell line N417: variant SCLC, no prior therapy*** | | | |
| Etoposide | (17)† | Etoposide | (8)† |
| Vincristine | (18)† | Doxorubicin | (68) |
| Doxorubicin | (23)† | Melphalan | (80) |
| Cisplatin | (56) | Vincristine | (82) |
| Melphalan | (61) | Cisplatin | (93) |
| CCNU | (> 100) | CCNU | (> 100) |
| ***Cell line H146: classic SCLC, prior therapy*** | | | |
| Etoposide | (66) | Melphalan | (54) |
| Melphalan | (70) | Etoposide | (68) |
| Doxorubicin | (70) | Doxorubicin | (76) |
| CCNU | (80) | Vincristine | (80) |
| Vincristine | (84) | Cisplatin | (90) |
| Cisplatin | (84) | CCNU | (> 100) |

*Rank order determined by % survival at 1× dose of drug as listed in table 1.
†Cell lines regarded as sensitive to these drugs.

## DISCUSSION

The development of individualized sensitivity tests for cancer chemotherapy that parallel tests for antibiotic

TABLE 4.—In vitro chemosensitivity of SCLC cell lines to a panel of relevant drugs*

| | Doxorubicin (μg/ml) | | | Cisplatin (μg/ml) | | | Etoposide (μg/ml) | | | Vincristine (μg/ml) | | | CCNU (μg/ml) | | | Methotrexate† (μg/ml) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 12 | 1.2 | 0.12 | 66 | 6.6 | 0.66 | 1250 | 125 | 12.5 | 8 | 0.8 | 0.08 | 100 | 10 | 1 | 200 | 20 | 2 |
| Established cell lines | | | | | | | | | | | | | | | | | | |
| Classic—no prior therapy | | | | | | | | | | | | | | | | | | |
| H209 | 2 | 2 | 36 | 1 | 6 | 35 | 0 | 14 | 56 | | | | | | | 7 | 27 | 36 |
| H187 | 5 | 49 | > 100 | 29 | 79 | 85 | 0 | 13 | 35 | | | | | | | | | |
| Classic—prior therapy | | | | | | | | | | | | | | | | | | |
| H69 | 31 | 55 | > 100 | 50 | > 100 | > 100 | 0.4 | > 100 | 97 | | | | | | | | | |
| H146 | 34 | 35 | 37 | > 100 | > 100 | > 100 | 0 | 6 | 38 | | | | | | | 10 | 22 | 35 |
| Variant—no prior therapy | | | | | | | | | | | | | | | | | | |
| N417 | 0 | 23 | 82 | 47 | 56 | > 100 | 0.4 | 17 | 84 | 14 | 18 | > 100 | 0.3 | > 100 | 90 | | | |
| Short-term cultures | | | | | | | | | | | | | | | | | | |
| H678 | 5 | 33 | > 100 | 71 | > 100 | > 100 | 0 | 65 | > 100 | 15 | 56 | 94 | | | | | | |

*Values in table are % survival.
†Continuous exposure; all other drugs exposed for 1 hr.

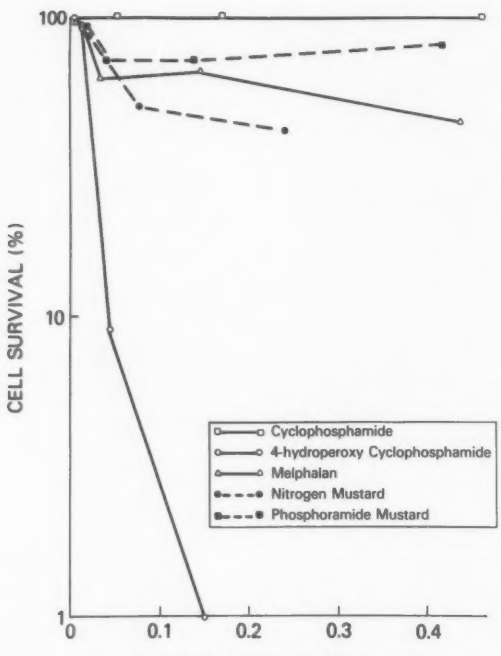


FIGURE 1.—Doses of the various cyclophosphamide derivatives or substitutes were compared on a mmol basis for activity against N417, a variant SCLC cell line known to be sensitive to a number of other drugs.

therapy has been a goal of researchers for > 30 years. It is a goal, however, that has remained elusive. Although numerous attempts have been made to analyze the effects of drugs on cell metabolism, cell morphology, radionuclide incorporation, and various aspects of cell membrane integrity, there has been no consensus that any of these assays can be utilized routinely in the clinical setting (reviewed in refs 4 and 23).

The growth of human cancers in nude mice with subsequent "in vivo" chemosensitivity testing is feasible but very expensive (24). To circumvent this problem, Bogden and others (25-27) have suggested that human tumor fragments placed in the subrenal space of mice will grow and their sensitivity to various drugs can be predicted by the differences in tumor growth between treated and untreated mice. Although clinical correlations appear reasonable (26), the assay is time- and resource-intensive and may not reflect actual tumor growth, as demonstrated by Bennett and co-workers (28). The major advantage of the assay lies in the preservation of cell-cell and cell-matrix interactions by the use of tumor fragments.

The clonogenic assay, popularized by Salmon et al (3), has become a standard for in vitro testing, despite its many technical limitations. There are numerous reports of its use in lung cancer, but the results suggest that it can be used only to test three or more drugs in approximately 20% of lung cancer cases (8-11). With the now routine isolation of cell lines from many patients with both SCLC and non-SCLC, it would appear that the limitations of testing 100 fresh samples to get 20 interpretable assays could be overcome, at least for the testing of new compounds. Unfortunately, despite being selected for "immortality" in the in vitro setting, most cell lines do not clone well in agarose (table 2). This may reflect the loss of cell-cell interactions or the absence of undefined nutrients, but the specific nature of the deficit is unclear. Nor is it clear that using conditioned or otherwise supplemented media will do more than select out a sensitive subpopulation of cells. Indeed, the technical aspects of the assay are relevant to the use of cell lines as well, with cell dissociation being a significant problem.

It was against this backdrop of an in vitro assay that could not be done in most clinical situations, nor done routinely even in the setting of continuous cell lines, that we chose to explore the novel dye-exclusion assay reported by Weisenthal and his colleagues (13). Dye-exclusion assays have been faulted for failure to distinguish normal from malignant cells, for lack of specificity, and for the inability to account for continued proliferation in surviving cell populations (29). Using a unique internal standard (the morphologically distinct DRBC) and a modified staining system, Weisenthal has reported the ability to analyze > 80% of clinical specimens and has reported preliminary clinical correlations (13). We have employed the assay in our panel of cell lines, both continuous and newly established, in order to establish its usefulness and to compare it to results obtained with the clonogenic assay.

The nonclonogenic, novel dye-exclusion assay can be performed on all cell lines we have tested and virtually all fresh clinical specimens. It is somewhat easier to perform than the clonogenic assay but more tedious to score. It is significantly faster than the clonogenic assay: 4 days versus 2-3 weeks. Results appear reasonably similar between the assays. The clinically refractory nature of non-SCLC is apparent in the low in vitro response rates. The variation between lines and the dose response patterns seen for individual lines suggest that this may be a powerful tool for testing new compounds. Sensitive cell lines such as the non-SCLC line H460 and the SCLC line H187 could be used to screen for new compounds, and the more refractory lines could be used as a better assessment of whether to move the agent to clinical trial. The ability to prepare short-term cultures from many clinical specimens would permit an increase in the 80% of specimens analyzable from fresh samples and reduce the need for immediate testing. Clinical correlations are badly needed for this assay; the NCI-Navy trials in SCLC and the Veterans Administration studies from California in non-SCLC should provide these results (39,40).

Another advantage to the Weisenthal assay is the degree to which the empirically derived drug doses

approximate the AUC for a given concentration of drug, as seen in table 1. Weisenthal et al (13) develop a dose-response curve for a new drug in two highly sensitive cell lines and test a drug near the $LD_{50}$ in a series of clinical specimens to obtain a "standard" concentration. This dose is then bracketed to provide a concentration-response curve, usually plus or minus a tenfold concentration. We adopted the concentrations derived by Weisenthal, but new drug testing and further studies of conventional agents could begin at the AUC concentration. Of interest in our results was the remarkable consistency with which the 10× dose of etoposide produced nearly total cell kill in almost every instance; 10× doses of doxorubicin and mitomycin had no comparable effect. Our analysis of the various in vitro substitutes for cyclophosphamide, which must be activated in vivo, suggests that the 4-hydroperoxycyclophosphamide derivative is the most active, as suggested by Colvin and Hilton (21).

The assay is not without problems, however. The variation in results is primarily due to problems in interpreting the slides. If the control or nonresponsive cell population is growing, the cells tend to reaggregate into large clusters, often > 200–500 cells. These cannot be analyzed for dye uptake. Most methods to reduce cell aggregation, including the one suggested by Weisenthal, are ineffective. The slides are tedious to review and require modest skill in interpreting cytological preparations. A reliable means of separating cells, which would not cause significant changes in dye uptake, would allow prompt automation of this procedure by flow cytometry (41).

In conclusion, it appears that the nonclonogenic dye-exclusion assay reported by Weisenthal is a rapid means of assessing in vitro drug sensitivity in most cell lines and clinical specimens and gives results comparable to those from clonogenic assay. Although clinical correlations await the completion of prospective trials of assay-directed therapy the assay can readily be used in cell lines to test new agents and drug-drug interactions.

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# Multivesicular Liposomes Containing Cytarabine Entrapped in the Presence of Hydrochloric Acid for Intracavitary Chemotherapy[1,2]

**Sinil Kim* and Stephen B. Howell[3]**

**For a full exploitation of the 1000-fold ip pharmacokinetic advantage obtained when cytarabine is administered ip, the drug exposure must be prolonged because the drug is a cell cycle phase-specific agent. Multivesicular liposomes made from nontoxic components were studied as biodegradable, slow-release microcapsules for intracavitary therapy. No attempt was made to increase efficacy by fusing liposomal membrane with that of tumor cells or by selective targeting of liposomes to cancer cells other than simple administration into a cavity. The presence of HCl with cytarabine at the time of encapsulation was found to decrease the leakage rate in human plasma dramatically. After ip administration of encapsulated cytarabine to mice, the total intracavitary drug concentration initially increased tenfold in 15 hours, then fell to the original concentration at 87 hours. The total amount of the drug in the peritoneal cavity decreased with a 21-hour half-life. All mice treated 24 hours after ip inoculation with $1 \times 10^6$ L1210 cells were cured when 32 mg/kg or 16 mg/kg of cytarabine in liposomes was given ip every 4 days for a total of three doses. Multivesicular liposomes do prolong intracavitary exposure to cytarabine and are efficacious in this model system. [Cancer Treat Rep 71:705–711, 1987]**

Many investigators in the past have attempted to direct cytotoxic drugs to the tumor location, to reduce systemic toxicity and increase tumor kill. One approach is the instillation of drugs directly into a tumor-containing cavity. This is particularly attractive for those cancers that grow and spread within confines of a cavity, such as ovarian carcinoma.

When a drug is instilled into an extravascular cavity, the concentration of the drug in the cavity relative to that in the plasma is determined by how rapidly the drug is cleared from the cavity relative to the drug's plasma clearance (1). The slower the intracavitary clearance and the higher the plasma clearance, the greater will be the cavity-to-plasma concentration ratio. The differential in the clearance rate is especially prominent for the peritoneal cavity and for those drugs that are metabolized rapidly in the liver ("first-pass" effect), because approximately 70% of small molecules leaving the cavity pass through the portal circulation of the liver (2–4). Specifically, human studies of ip cytarabine have shown that the peritoneal:plasma concentration ratio was almost 1000:1 (5).

To achieve optimal cancer cell kill with a cell cycle phase-specific drug like cytarabine, two major requirements need to be met: first, the cancer cells must be exposed to a high concentration of the drug without doing irreversible harm to the host; and second, the tumor must be exposed for a prolonged period of time so that all or most of the cancer cells have attempted to synthesize DNA in the presence of cytarabine. Ip administration of cytarabine permits a very high local cytarabine concentration without severe harm to the host; but the second requirement of long drug exposure is problematic because of rapid drug clearance from the peritoneal cavity, with half-lives of 60–210 minutes (5). Therefore, some sort of a slow-release form of the drug is needed. We have explored the use of multivesicular liposomes as biodegradable, nontoxic microcapsules to achieve this end.

Multivesicular liposomes (6) are distinct from unilamellar (7,8) and multilamellar (9,10) liposomes in that the multivesicular liposomes enclose multiple nonconcentric internal chambers, the capture efficiency is high, and the average size of liposomes and their internal

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chambers can be adjusted. Multivesicular liposomes are especially well suited to serve as slow-release microcapsules for hydrophilic molecules, such as cytarabine, because the molecules entrapped in inner chambers have to cross multiple bilayer membranes to leak out and the average number of internal chambers can be easily adjusted to tailor-make the rate of release.

## MATERIALS AND METHODS

### Materials

Cytarabine (Cytosar-U) was a gift from the Upjohn Co, Kalamazoo, MI; dioleoyl lecithin, dipalmitoyl phosphatidylglycerol, and cholesterol were purchased from Avanti Polar-Lipids, Inc, Birmingham, AL; triolein and L-lysine, free base, were procured from Sigma Chemical Co, St. Louis, MO; nanograde chloroform was from Mallinckrodt Inc, Paris, KY; and all were used without further purification. [$^3$H]cytarabine was purchased from Amersham Corp, Arlington Heights, IL, and the purity was checked by high-performance liquid chromatography (HPLC). $BDF_1$ and DBA/2 mice were from Simonsen Laboratories, Gilroy, CA; vortex mixer was from American Scientific Products, catalogue No. S8223-1, McGaw Park, IL.

### Synthesis of Multivesicular Liposomes

For each batch of liposomes prepared, 1 ml of 20-mg/ml cytarabine solution (with pH adjusted appropriately with 1 N HCl) was added into a 1-dram vial containing 9.3 μmols of dioleoyl lecithin, 2.1 μmols of dipalmitoyl phosphatidylglycerol, 15 μmols of cholesterol, and 1.8 μmols of triolein and 1 ml of chloroform. The vial was attached to the head of the vortex mixer and shaken at the maximum speed for 6 minutes. Half of the resulting "water-in-oil" emulsion was squirted rapidly through a narrow-tip Pasteur pipette into 1-dram vials, each containing 2.5 ml of water, glucose (3.2 g/100 ml), and free-base lysine (40 mM), and then shaken on the vortex mixer for 3 seconds at "5" setting to form chloroform spherules. The chloroform spherule suspensions in the two vials were transferred into the bottom of a 250-ml Erlenmeyer flask containing 5 ml of water, glucose (3.2 g/100 ml), and free-base lysine (40 mM). A stream of nitrogen gas at 7 L/minute was flushed through the flask to evaporate chloroform over 10–15 minutes at 37°C. The liposomes were then isolated by centrifugation at 600 × *g* for 5 minutes, and washed with phosphate-buffered saline (NaCl, 8 g/L; $Na_2HPO_4$, 0.92 g/L; $KH_2PO_4$, 0.2 g/L; pH 7.4) thrice. The liposomes were used within 24 hours. Size distribution study was done as described previously (6). Previous investigation has shown that no chloroform is detectable by gas chromatography (6).

### HCl Retention Studies

The amount of HCl remaining inside liposomes was estimated by washing liposomes with unbuffered 0.9% (wt/vol) NaCl solution three times, addition of 10× volume of distilled water to the pellet, and sonication with probe sonicator to lyse liposomes. The pH of the resulting solution was measured and the internal liposomal pH prior to lysis was estimated by taking the dilution factor into account.

The extent of cytarabine ionization was estimated in situ by mounting liposome pellet on 0.2-mm path-length quartz cell (Hellma, Jamaica, NY) and obtaining the UV absorption spectrum. The maximal absorption of ionized cytarabine at a pH of 2 was 281 nm and that of un-ionized drug at a pH of 7 was 273 nm.

### Drug Release Studies

Cytarabine release studies were done with tritium-labeled drug by adding at least 100 volumes of phosphate-buffered saline, human ascitic fluid obtained from a patient with advanced ovarian carcinoma, or blood bank plasma, to the washed liposome pellets. The liposome suspension was placed in a syringe, 0.01% sodium azide was added to inhibit growth of microorganisms, air was excluded from the syringe to reduce oxidation of lipids, and the syringe was placed on a rotary shaker (G10 Gyrotory Shaker, New Brunswick Scientific Co, New Brunswick, NJ) in a warm room kept at 37°C. At appropriate time points, aliquots were removed from the syringe, liposomes were separated from the suspending medium by centrifugation in Eppendorf microfuge for 5 minutes, and the radioactivity in the liposome pellet was compared against that at time zero point and expressed as percentage remaining in liposomes. For the release studies in 100% human plasma, liposomes were separated from the suspending medium by ultrafiltration through YMT membrane (Amicon Corp, product No. 4104, Danvers, MA) because the liposomes floated up in plasma. The radioactivity in the ultrafiltrate was compared against that in an equal volume of prefiltration plasma to calculate the percentage of cytarabine released. The pH of plasma did not change significantly at the end of the leakage studies.

### In Vivo Studies

Ip pharmacokinetic studies were done on male $BDF_1$ mice weighing 16–25 g. A group of mice were injected ip with 1 mg of free or encapsulated drug, or glucose-containing "empty" liposomes in 1 ml of 0.9% NaCl solution. At appropriate time points, animals were killed by cervical dislocation and peritoneal fluids were first collected into capillary tubes of defined volumes. Then the peritoneal cavities were washed out thoroughly with 1–2 ml of 0.9% NaCl solution thrice. "Free" cytarabine concentrations were measured after removal of liposomes and cells from the peritoneal fluids by centrifugation. Tetrahydrouridine (40 μM final concentration) was added to prevent degradation of cytarabine, and the specimen was kept frozen at −20°C until assay. Fluids containing liposomes were diluted at least ten-fold with distilled water and sonicated for 10 seconds with a probe

sonicator ("50" setting, low power, on Biosonik IV, VWR Scientific, San Francisco, CA) to disrupt liposomal membranes before analysis. Cytarabine was quantitated on a Waters and Associates (Milford, MA) HPLC with 280-nm detector as previously described (5), on C18 reverse-phase Radial-Pak cartridge column, with 5 mM potassium phosphate buffer at a pH of 7.0 as mobile phase, running at a rate of 2.0 ml/minute. Retention time for cytarabine was 5 minutes and that for the major metabolite, uracil arabinoside, was 7 minutes.

For toxicity and efficacy studies, $BDF_1$ mice were inoculated with $1 \times 10^6$ L1210 cells ip on Day 0, and treated ip with liposomes suspended in phosphate-buffered saline on Days 1, 5, and 9, or on Day 1 only. The amount of cytarabine in liposomes was measured on a spectrophotometer (UVIKON 810, Kontron International, Zurich) at 280 nm in 100% methanol containing 10 mM sodium acetate. The absorbance was linear with respect to concentration in the range measured and the liposomal lipids did not absorb at the wave-length used. The liposome suspension in phosphate-buffered saline was diluted to contain 1.3 mg/ml of cytarabine and appropriate volumes were injected for various dosage lvels.

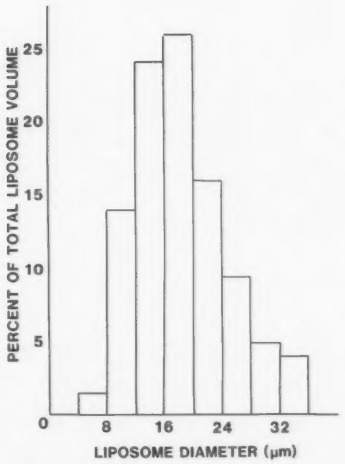


FIGURE 2.—Volume-adjusted size distribution of multivesicular liposomes containing cytarabine. Diameters of liposomes were measured in groups of 4-μm intervals from a photomicrograph. The number of liposomes in each size group was multiplied by the cube of the diameter to obtain relative volumes of each size category and then divided by the sum of the relative volumes of all liposomes to obtain percent of total liposome volume represented by each size category.

## RESULTS

### Size Distribution and Efficiency of Cytarabine Encapsulation

Figure 1 shows a photomicrograph of multivesicular liposomes containing cytarabine at a pH of 1.1. Figure 2 shows percent of the total liposome preparation volume contributed by liposomes of increasing diameter. The average volume-adjusted size of the liposomes (± standard deviation of the distribution) was 19.4 ± 6.5 μm. Percentage of capture was 59 ± 7% ($n = 12$) and capture volume was 36 ± 4 μl/mg of total lipids used.

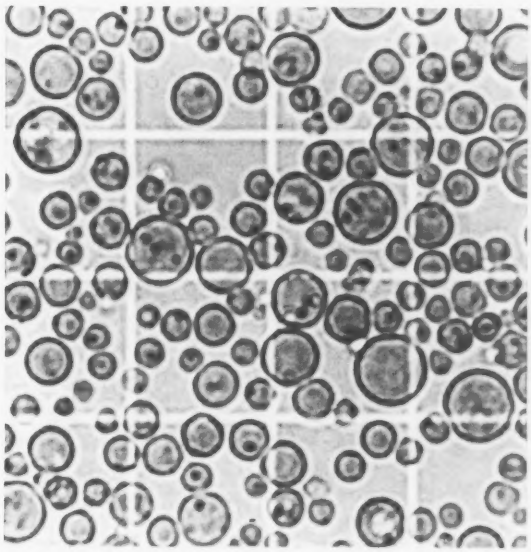

FIGURE 1.—Photomicrograph of multivesicular liposomes containing cytarabine entrapped in the presence of HCl (initial pH of 1.1). The small squares of the hemacytometer lines are 50 × 50 μm.

### HCl Retention Studies

The lysis of 0.1 ml of liposome pellet (prepared in the presence of HCl with the initial pH of 1.1) with 1 ml of distilled water gave a pH of 6, indicating internal liposomal pH of about 5 prior to lysis. An identical experiment, except for replacement of HCl with $H_2SO_4$ (initial pH of 1.1), gave a pH of 3, indicating internal liposomal pH of approximately 2 prior to lysis. The in situ ultraviolet spectrum of the former liposomes (HCl, initial pH 1.1) mounted on 0.2-mm cell resulted in maximum absorption at 273 nm, and the latter liposomes ($H_2SO_4$, initial pH 1.1) gave maximum absorption at 280 nm. Liposomes prepared in the absence of HCl (initial pH, 7.0) gave identical spectrum as the former liposomes (HCl, initial pH 1.1).

### Release Studies

When liposomes made in the absence of HCl were suspended in phosphate-buffered saline (pH 7.4), the release of cytarabine demonstrated first-order kinetics with a half-life of about 11 days (fig 3). The addition of HCl to cytarabine at the time of encapsulation did not significantly affect the release kinetics when liposomes were suspended in phosphate-buffered saline. However, leakage studies in plasma (fig 4) and ascitic fluid (fig 5)

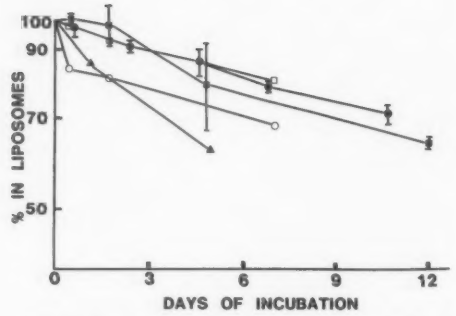


FIGURE 3.—Release of cytarabine from multivesicular liposomes suspended in phosphate-buffered saline (pH 7.4). Initial pH at the time of encapsulation was: pH 1.1 (●); pH 2.0 (○); pH 3.7 (□); pH 4.6 (■); and pH 7.0 (▲). Each point is the mean and the standard deviation from 3 experiments for pH 1.1 and 4.6. The rest are from single experiments. Note logarithmic scale of ordinate.

showed that the release of cytarabine from multivesicular liposomes was markedly influenced by the presence of HCl with cytarabine at the time of encapsulation. For example, when the initial encapsulation pH was 1.1,

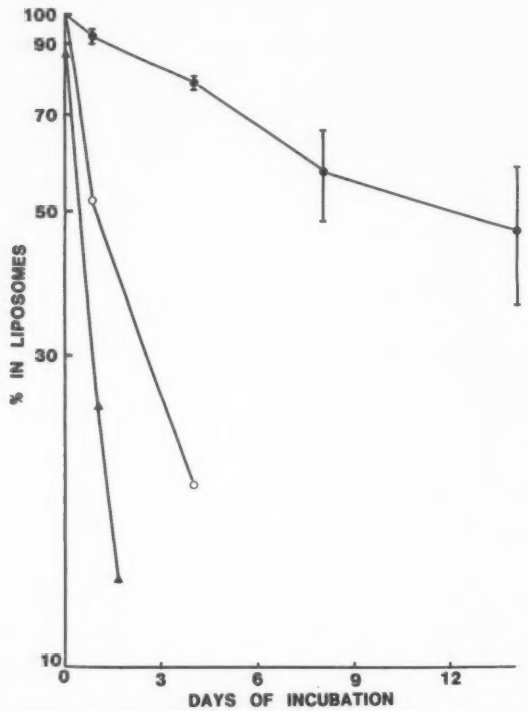


FIGURE 4.—Release of cytarabine from multivesicular liposomes suspended in 100% human plasma. Initial pH at the time of encapsulation was: pH 1.1 (●); pH 4.6 (○); and pH 7.0 (▲). Each point is the mean and the standard deviation from 3 experiments for pH 1.1. The rest are from a single experiment. Note logarithmic scale of the ordinate.

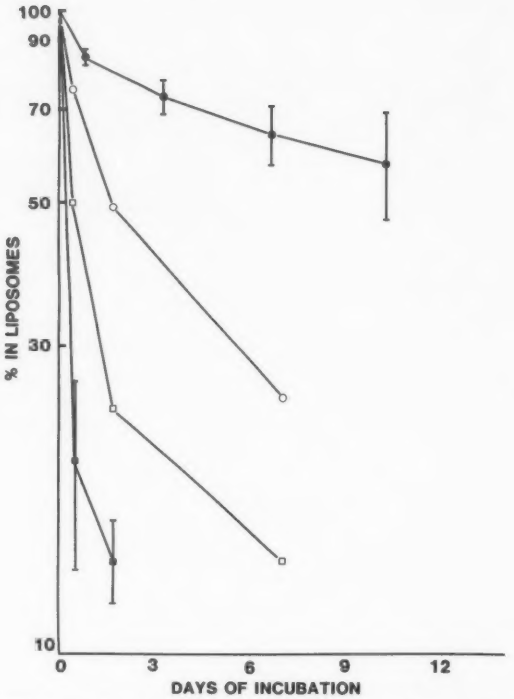


FIGURE 5.—Release of cytarabine from multivesicular liposomes suspended in 100% human ascitic fluid. Internal pH at the time of encapsulation was: pH 1.1 (●); pH 2.0 (○); pH 3.7 (□); and pH 4.6 (■). Each point is the mean and the standard deviation from 3 experiments for pH 1.1 and 4.6. The rest are from single experiments. Note logarithmic scale of the ordinate.

half-life of leakage in plasma was 12 days, whereas it was only 12 hours at encapsulation pH of 7.0. Storage of liposomes at 4°C without agitation resulted in < 10% leakage at 3 months.

### Intraperitoneal Pharmacokinetics

Following ip injection of free cytarabine, the ip concentration decreased exponentially with a half-life of 16 minutes. The ip half-life of cytarabine encapsulated in the absence of HCl (initial encapsulation pH = 7.0) was 2–3 hours (fig 6a).

For the cytarabine encapsulated in the presence of HCl (initial pH = 1.1), the drug remained in the peritoneal cavity much longer. Following ip injection, total concentration of cytarabine (free plus entrapped) in the peritoneal cavity was found to increase tenfold over the first 15 hours (fig 6b). During this period of time, the amount of fluid present in the cavity was observed to decrease, with simultaneous increase in turbidity of the fluid. At 87 hours after the ip injection, the total cytarabine concentration returned to the original level, and at 130 hours it was about one-tenth of the original concentration. The "free" cytarabine concentration (fig 6c) ob-

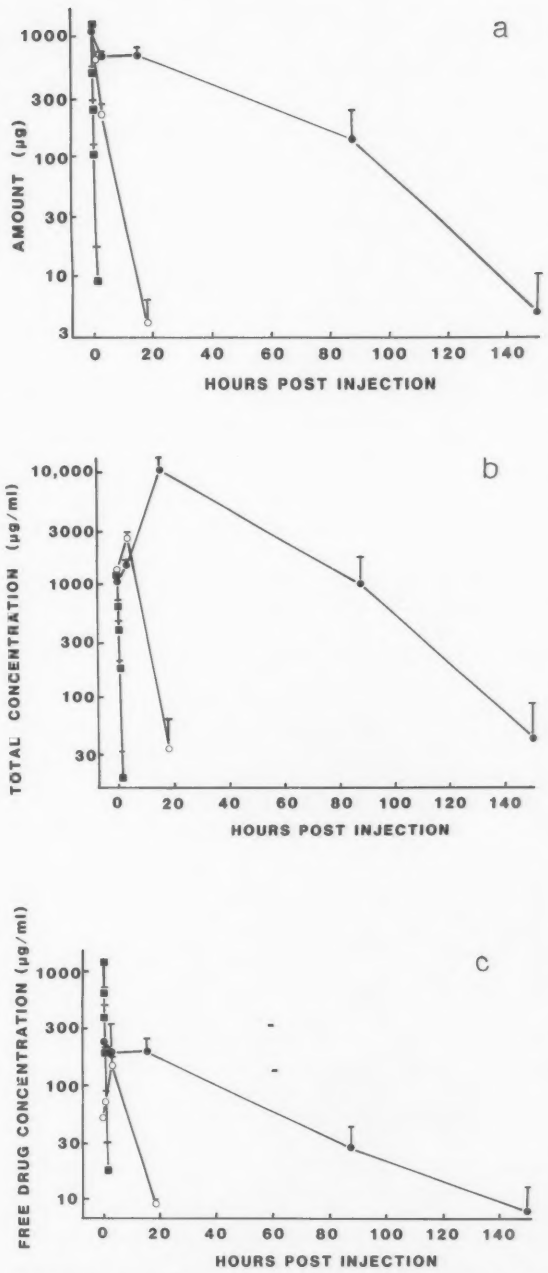


FIGURE 6.—Ip concentrations and amounts of cytarabine after ip administration of 1 mg of cytarabine as a free drug (■), encapsulated into multivesicular liposomes without the presence of HCl (○), and encapsulated in the presence of HCl, initial pH 1.1 (●). (a) Total cytarabine amount in μg; (b) total cytarabine concentration in μg/ml; (c) "free" cytarabine concentration in μg/ml. Each point represents the mean and standard deviation of results from 3 mice. Note logarithmic scale of the ordinates.

tained after removal of liposomes by centrifugation was about one-fifteenth of the total drug concentration, and the pharmacokinetic curve for the free drug approximately paralleled that of the total concentration. The total amount (not concentration) of ip cytarabine obtained by washing the peritoneal cavity was found to decrease with a half-life of about 21 hours (fig 6a).

### Efficacy Studies

Based on the results of the pharmacokinetic studies showing that a significant amount of the cytarabine persisted in the peritoneal cavity even after 4 days when the drug is encapsulated in the presence of HCl, efficacy studies were done by treating mice by the ip route every 4 days for three doses. Figure 7 shows the survival curves for mice treated with varying doses of cytarabine encapsulated in multivesicular liposomes. Those mice treated with 48 mg/kg × 3 of cytarabine in liposomes all died from toxicity 3–6 days after the third dose. Mice treated with lower doses × 3 were apparently all cured, surviving > 60 days after implantation of $1 \times 10^6$ L1210 cells.

In a separate experiment, mice were treated with a single-dose schedule. Four of five mice survived 30 days at dosages of 63 mg/kg and 126 mg/kg, but 60-day survival was two of five in each dosage group. Treatment with "empty" liposomes gave no increase in survival.

## DISCUSSION

Even though cytarabine in liposomes was extensively studied since the mid-1970s (11), its use in humans has not yet been reported. This is probably because of the fact that the earlier research has been directed mainly toward systemic administration of the preparations via iv route, which results in most of the drug and liposomes accumulating in liver and spleen. Ip administration of cytarabine in liposomes has been utilized early in animal models, but this route was mainly for the convenience of administration, rather than for the explicit purpose of using the system as a model for human ip therapy. Perhaps this is also the reason that the detailed ip pharmacokinetics of cytarabine in liposomes have not yet been reported even though extensive pharmacokinetic studies have been done for the distribution of the drug in various other tissues (12,13).

In previous studies, leakage of the drug from liposomes has been too rapid in biological fluids to be clinically useful as slow-release depot. For example, 60.3% leaked out at 4 hours from multilamellar liposomes (14), 30% leaked out by 24 hours from reverse-phase evaporation liposomes (15), and 50% leaked out by 21 and 28 hours from small unilamellar and reverse-phase evaporation liposomes, respectively (16). These leakage rates are quite inferior to that in this study. Positively charged liposomes, using stearylamine as one of the membrane lipids, have demonstrated slow release rates of 3%–4% over 24 hours in serum (17). However, stearylamine is

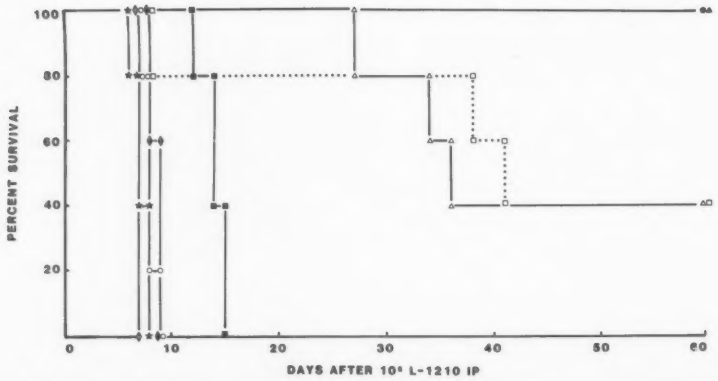


FIGURE 7.—Therapeutic effects of cytarabine in multivesicular liposomes. Mice were inoculated with $1 \times 10^6$ L1210 cells ip on Day 0, and treated ip with cytarabine-containing liposomes suspended in phosphate-buffered saline either on Day 1 (single-dose treatment) or on Days 1, 5, and 9 (3-dose treatment). Symbols are as follows: 48 mg/kg × 3 of encapsulated cytarabine (■); 32 mg/kg × 3 of encapsulated drug (●); 16 mg/kg × 3 of encapsulated drug (▲); no treatment (★); free cytarabine at a nontoxic dose of 48 mg/kg × 3 (◆); mice treated with liposomes containing glucose (◇); 63 mg/kg × 1 of encapsulated drug (△); 126 mg/kg × 1 of encapsulated drug (□); and 178 mg/kg × 1 of encapsulated drug (○).

not a natural component of mammalian cells, and is toxic to cells at high concentrations (18).

With multivesicular liposomes synthesized from nontoxic, biodegradable membrane components, we have been able to increase the half-life of cytarabine release in biological fluids to the range of weeks. It is not clear at this time how the presence of HCl at the time of encapsulation increases the retention of cytarabine in multivesicular liposomes. It apparently is not due to ionization of the cytarabine at low pH by the HCl since HCl is very soluble in organic solvents (19) and the permeability of molecular HCl through lipid bilayer membranes is exceedingly high (20), resulting in almost complete leakage of HCl by the time synthesis and washings of liposomes are completed.

It was a novel finding that the concentration of cytarabine in the peritoneal cavity actually rose during the first 15 hours and stayed above the original concentration for a period of 87 hours. We attribute this initial rise in concentration to a differential clearance of the phosphate-buffered saline versus the liposomes. The free fluid surrounding the liposomes is rapidly absorbed whereas the liposomes themselves are not. Since the amount of cytarabine in the liposomes stays about the same, the total amount/volume (ie, concentration) of the drug increases with time. This fact may have a positive therapeutic implication, in that the cytotoxic effect on tumor cells is usually concentration dependent, ie, the higher the concentration, the greater the antitumor effect.

The results of the ip pharmacokinetic study permitted selection of a rational dose schedule. Every-4-day dosing for a total of three doses was sufficient to cure 100% of mice when the treatment was begun 24 hours after implantation of $1 \times 10^6$ L1210 cells. Previous studies by other investigators using other types of liposomes have also shown long-term survivors, but the long-term survival was measured at 30 days rather than at 60 days (15,16) and some of the long-term survivors could have died between 30 and 60 days as indicated by our data on single-dose treatment (fig 7). In addition, previous studies did not explore multiple-dose schedule, which would more closely approximate the way this drug would be given to human cancer patients (ie, single-dose treatments are not usually given to patients).

We do not believe that the liposomes are doing anything more than serving as a slow-release depot. We and previous investigators (16) have no evidence for a selective targeting of liposomally entrapped drug to tumor cells, nor for fusion of the tumor cell membrane with that of the liposomes. Therefore, if a continuous infusion of cytarabine into the peritoneal cavity were given for an identical period of time, the expected results would be similar to what we have found with the use of liposomes. In fact, some cures have been achieved previously by very frequent administration of free drug (21). Nevertheless, the advantage of using a few doses of depot preparation, instead of every-3-hour dosing for 32 total injections or continuous infusion, is obvious.

We have not examined the penetration of the drug into tumor nodules. We expect that the cytarabine molecules would not penetrate very deeply into tumor nodules, and therefore would not work well against bulky tumors. Indeed, human clinical studies done at our institution have shown that the size of the ovarian cancer at the time of ip therapy has major effect on the outcome, and that in patients with small-volume disease, survival is excellent after treatment with ip cytarabine and cisplatin, ie, 69% survival at 4 years (22).

Even though the L1210 is not strictly confined to the peritoneal cavity after implantation in the cavity, the bulk of the cancer cells still remain in the cavity 24

hours after implantation when the chemotherapy is started (23). In any case, the imperfection in this tumor model does not invalidate the pharmacokinetic studies that showed prolonged exposure of the peritoneal cavity to the cytotoxic levels of cytarabine.

Based on the in vitro leakage studies, ip pharmacokinetic studies, and ip L1210 efficacy studies, we believe that the utility of multivesicular liposomes as slow-release depot is amply demonstrated in this model system. In comparison to other types of liposomes, the multivesicular liposomes are especially suitable for clinical applications in humans because of the large internal aqueous spaces with highly efficient incorporation of hydrophilic drugs, the ease of scale up, and the stability in storage. These multivesicular liposomes may also prove clinically useful not only for the treatment of ovarian carcinoma, but also for meningeal leukemia and other tumors located in extravascular cavities.

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# Phase II Evaluation of Teniposide and Ifosfamide in Refractory Adult Acute Lymphocytic Leukemia: A Southwest Oncology Group Study[1,2]

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**The Southwest Oncology Group undertook a phase II study of teniposide (VM-26) and ifosfamide in refractory adult acute lymphocytic leukemia. The 49 evaluable patients were heavily pretreated; 15 of these had received only one prior induction attempt. Two treatment regimens were used. Eighteen patients received VM-26 (30 mg/m$^2$) on Days 1-5 and ifosfamide (1000 mg/m$^2$) by continuous iv infusion on Days 1-5. When acceptable toxicity was observed, the dose of VM-26 was increased and 30 patients received VM-26 (50 mg/m$^2$) on Days 1-5 in addition to ifosfamide. Complete remission (CR) was observed in two patients who received the lower-dose VM-26 regimen and in six patients who received the higher-dose regimen. Of the eight patients who achieved CR, six were found among the 15 patients who had received only one prior induction therapy. Hematologic toxicity was the major toxic effect observed, with 41 patients (84%) having wbc counts < 1000/μl during induction therapy. Nonhematologic toxicity was dose-limiting in seven patients for the following reasons: hematuria (four patients), neurological disturbance (one), and mucositis (two). The combination of VM-26 and ifosfamide is capable of producing CR in refractory acute lymphocytic leukemia in adults with manageable toxicity. Inclusion of the combination in a multidrug consolidation regimen for newly diagnosed patients is an appropriate avenue for further study. [Cancer Treat Rep 71:713-716, 1987]**

In contrast to children with acute lymphocytic leukemia (ALL), for whom 70% cure rates are reported, adults with ALL face a poorer prognosis. Remission induction therapy in adult ALL results in complete remission (CR) rates of 70%-80% in most contemporary series (1). However, in a large series of adult patients (2-5) median duration of CR is 5.9-20 months, median survival is 17-34 months, and 5-year relapse-free survival is 25%-32%. Clearly, there is need for progress in the development of new combinations of agents and regimens for the treatment of adults with this disease.

Teniposide (VM-26) and ifosfamide are an attractive potential combination to study in ALL because each is only mildly myelosuppressive and each has demonstrated activity in lymphoproliferative neoplasms (6-10). VM-26 is active as a single agent (8) and in combination with cytarabine (9) in childhood ALL. In an earlier Southwest Oncology Group study (11), ifosfamide was combined with doxorubicin using full normal doses of each drug. Toxicity was acceptable and eight of nine patients with previously treated ALL achieved CR.

As reported in this paper, the Southwest Oncology Group did a phase II pilot study of the combination of VM-26 and ifosfamide in the treatment of adults with refractory ALL.

## MATERIALS AND METHODS

### Patients

Previously treated adult patients with ALL who had failed to respond to initial chemotherapy or had relapsed following standard chemotherapy were eligible for this study. Criteria for entry in the protocol included the following: (*a*) bone marrow examination with absolute infiltrate of 50% leukemic cells or, if the absolute infiltrate was 30%-49%, evidence of progressive disease prior

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to entry; (*b*) serum creatinine ≤ 2 mg/dl; (*c*) serum bilirubin ≤ 2 mg/dl; (*d*) < 10 rbcs/high-power field of centrifuged urinary sediment; (*e*) Southwest Oncology Group performance status of 3 or greater, where patients were not completely disabled, were able to carry on some self-care, and were not fully confined to a bed or a wheelchair; and (*f*) written informed consent in accordance with institutional and Food and Drug Administration guidelines.

### Treatment

Induction therapy consisted of up to three courses of the combination. Ifosfamide (1000 mg/m$^2$/day) was given by continuous infusion on Days 1-5 in 3 L of 5% dextrose in water/24 hours. While receiving ifosfamide therapy, each patient was given 500 mg of ascorbic acid orally every 6 hours and a daily urinalysis was done. If microscopic hematuria was > 10 rbcs/high-power field, ifosfamide was discontinued for at least 24 hours and restarted only when the urine had < 10 rbcs/high-power field. VM-26 was given at two initial dose schedules on the protocol. VM-26 was given daily over 45 minutes on Days 1-5. Eighteen patients received an initial dose of VM-26 (30 mg/m$^2$/day) for 5 days. Since only moderate hematologic toxicity was observed, the protocol was amended and patients received a higher VM-26 dose (50 mg/m$^2$/day) for Days 1-5 in combination with the ifosfamide.

A bone marrow aspirate or biopsy was required on Day 15. For patients who did not achieve CR of their disease after one course of chemotherapy, re-treatment was allowed on Day 15 if the bone marrow examination showed > 50% blasts. If hypoplasia occurred and bone marrow recovery was delayed, re-treatment could be given on Day 21, or later.

For subsequent induction courses, doses were adjusted for nonhematologic toxicity as follows: if any nonhematologic toxicity graded severe or worse by objective criteria was observed, doses were decreased by 25%; otherwise, doses were increased by 25%. Patients received no further protocol treatment if there was no reduction in bone marrow leukemic infiltration after two courses or if potentially fatal nonhematologic toxicity occurred.

In Southwest Oncology Group criteria CR is defined as bone marrow examination showing essentially normal-appearing granulopoiesis, erythropoiesis, and thrombopoiesis except for morphologic changes definitely attributed to the medication employed. The sum of cells individually identified as prolymphocytes and lymphoblasts must be < 10% of the total nucleated cells and there must be < 20% lymphocytes in the marrow. Additionally, the peripheral blood must show the hemoglobin to be > 11 g/dl, with no transfusion during the preceding 2 weeks. The circulating granulocyte count level must be > 1000/$\mu$l but not > 9000/$\mu$l. There must be no blast forms in the peripheral blood and the platelet count must be > 100,000/$\mu$l. Also, there must be no physical findings to suggest leukemia infiltration of the spleen, lymph nodes, or other organs.

Patients who achieved CR received treatment until relapse. Courses were given every 28 days and consisted of VM-26 at 25 mg/m$^2$/day over 45 minutes on Days 1-5 and ifosfamide at 750 mg/m$^2$/day over 1-2 hours on Days 1-5.

## RESULTS

Fifty-three patients were entered in the study. Two patients were ineligible since one patient had bilirubin > 2 mg/dl at study entry and one had lymphoma. Table 1 shows pretreatment characteristics.

Fifty-one patients met criteria for eligibility to be treated with the combination of VM-26 and ifosfamide, but one refused protocol therapy. Eighteen patients received an initial dose of VM-26 at 30 mg/m$^2$/day × 5 and are evaluable for toxicity and response. Thirty-two patients received the increased induction dose level of VM-26 at 50 mg/m$^2$/day × 5 but one patient had insufficient laboratory data obtained for evaluation to be made of response and toxicity. The lower part of table 1 summarizes pretreatment characteristics of the eligible patients.

Table 2 shows response characteristics at the lower dose level of VM-26. Of the 18 evaluable patients, two

TABLE 1.—Pretreatment characteristics

| | | VM-26 dose (mg/m$^2$) | |
|---|---|---|---|
| | Total | 30 | 50 |
| No. entered | 53 | 21 | 32 |
| Ineligible | 2 | 2 | 0 |
| Not evaluable | 2 | 1 | 1 |
| Evaluable | 49 | 18 | 31 |
| Median age in yrs (range) | | 23 (18-56) | 32 (13-78) |
| No. of males (%) | | 11 (58) | 18 (58) |
| No. of whites (%) | | 15 (79) | 26 (84) |
| Prior therapy (eligible patients) | | | |
| Median No. of prior induction attempts | | 3 | 2 |
| No. with prior induction (%) | | 3 (16) | 12 (38) |

TABLE 2.—Response: VM-26 (30 mg/m$^2$)

| | No. of patients (%) |
|---|---|
| Evaluable | 18 (100) |
| CR | 2 (11) |
| Early death (before Day 15) | 2 (11) |
| No response (1 course) | 5 (28) |
| No response (2 courses) | 9 (50) |

TABLE 3.—Response: VM-26 (50 mg/m$^2$)

| | No. of patients (%) |
|---|---|
| Evaluable | 31 (100) |
| CR | 6 (19) |
| Early death (before Day 15) | 2 (6) |
| No response (1 course) | 6 (19) |
| No response (2 courses) | 17 (55) |

achieved CR. The durations of CR were 69 and 136 days. The patient whose response lasted 136 days refused chemotherapy after induction and thus had unmaintained remission. Both patients who achieved CR had had one prior induction therapy. Of the 18 patients treated at this dose level, there were only three who had had only one prior induction therapy. Two of those three patients achieved CR. Two patients died of sepsis at Days 3 and 11. Of the five patients who received only one course and did not achieve CR, two died of sepsis at Days 19 and 25. One patient had reversible coma and received no further therapy because of toxicity. The cause of coma was attributed to ifosfamide. One patient refused further therapy after one course. One investigator did not re-treat. Of the nine patients who received two full induction courses without achieving CR, one patient had marrow aplasia with < 5% marrow cellularity, but leukemic cells repopulated the marrow. In three patients there was decrease in marrow and peripheral leukemic blasts but failure to achieve marrow aplasia. In five patients, there was no decrease in peripheral or marrow leukemic blasts despite two induction courses.

Table 3 shows response to the combination at the higher dose of VM-26. There were six patients who achieved CR. The durations of CR were 23, 28, 42, 101, 140, and 292+ days. Four of the six patients who achieved CR had had only one prior induction and the other two patients who achieved CR had received four and five prior inductions. Of the 31 evaluable patients treated at this higher dose level, 12 had had only one prior induction and four of those 12 achieved CR. There were two early deaths at Days 7 and 8 due to sepsis and hemorrhage. Of the six patients who received only one course and did not achieve CR, there were two who refused further therapy, one who developed hemorrhagic cystitis and was not re-treated, one whose investigator did not re-treat, and two who died of sepsis at Days 28 and 32 without re-treatment. There were 17 patients who received two full courses of therapy and failed to achieve CR. In four of the 17 patients, marrow aplasia occurred, but leukemic cells repopulated the bone marrow. In ten of the 17 patients there was decrease in marrow or peripheral leukemic blast cells but failure to achieve aplasia. In three of the 17 patients there was no decrease in marrow or peripheral blasts.

Forty-nine patients were evaluable for toxicity. Hematologic toxicity was the major toxic effect that we observed. Of the 49 evaluable patients, 41 (84%) had wbc counts < 1000/$\mu$l during induction therapy and 38 (78%) had platelet counts < 50,000/$\mu$l.

Nonhematologic toxicity observed in the 49 evaluable patients included hematuria (four patients), neurological disturbance (one), and mucositis (two). Two patients developed gross hematuria attributed to ifosfamide which abated when the drug was stopped. In two patients, microscopic hematuria observed during induction therapy resulted in delay of administration of ifosfamide. One patient developed mental confusion which progressed to stupor and was attributed to ifosfamide. These symptoms reversed and the patient's mental status became normal when the drug was stopped. Two patients treated with the higher dose of VM-26 developed severe mucositis attributed to that drug.

## DISCUSSION

Our evaluation of 49 adult patients with ALL in relapse with two dosage levels of VM-26 in combination with ifosfamide was accomplished with acceptable toxicity. Severe hematologic toxicity occurred in most patients. In treating refractory ALL, the investigators expected severe hematologic toxicity with the combination and found it manageable. There were eight patients who died between Days 3 and 32 of sepsis or hemorrhage before completing the prescribed two courses on study, but we do not believe this to be an excessive rate in a group of patients who were heavily pretreated and had refractory disease. Severe nonhematologic toxicity occurred in five patients manifesting gross hematuria, mucositis, or stupor. Thus, dose-limiting nonhematologic toxicity occurred in 10% of the evaluable patients.

The combination of VM-26 and ifosfamide does have activity in adults with refractory ALL. Of 31 evaluable patients who received the combination with the higher dose level of VM-26, six (19%) achieved CR. Of 18 patients who received the combination with the lower dose level of VM-26, two (11%) achieved CR. Most of the evaluable patients had been heavily pretreated. Of the 49 evaluable patients, 34 had received more than one prior induction regimen. Of the eight patients who achieved CR, six were found among the 15 who had received just one prior induction.

In a prior evaluation (11), the Southwest Oncology Group treated nine ALL patients with ifosfamide and doxorubicin and eight of the nine achieved CR. Rodriguez et al (7) reported 12 patients treated with single-

agent ifosfamide and there were four CRs and one partial response. VM-26 has shown activity in combination with cytarabine in children with ALL (9) and remissions were achieved with the combination in patients who had previously failed on cytarabine. It is not possible to accurately compare the results we obtained with the combination of VM-26 and ifosfamide and the above-noted results because in our evaluation we did not randomize the combination with a single agent or another combination.

It is not possible to determine which agent was the more effective in the combination. When the dose of VM-26 was increased, there was a higher percentage of CR and a lower percentage of patients who failed to show a decrease in marrow or peripheral blasts. However, this was not a randomized comparison between the two doses of VM-26, and the group receiving the higher dose, in fact, included a greater proportion of patients with only one prior induction regimen, among whom six of the eight CRs occurred. Therefore, we cannot conclude from the present study that the higher dose of VM-26 produced a higher response rate.

A possible avenue for further investigation of the combination would be to study its use in less heavily treated adults with ALL. By adding another active agent to this combination, a more effective salvage regimen might be produced for use in ALL in relapse. Since the majority of newly diagnosed adults with ALL cannot expect cure with current standard chemotherapy, there is a need for new treatment plans in this disease. We have demonstrated the safety and activity of the combination of VM-26 and ifosfamide. Inclusion of the combination in a multidrug consolidation regimen for newly diagnosed ALL in adults is an appropriate avenue for further study.

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# Scheduling of Vincristine: Drug Accumulation and Response of Xenografts of Childhood Rhabdomyosarcoma Determined by Frequency of Administration[1,2]

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**Vincristine (VCR) is an effective agent in the treatment of childhood rhabdomyosarcoma. Clinically, schedules differ in frequency of administration. To determine the influence of administration frequency, accumulation of VCR in xenografts of human rhabdomyosarcoma has been evaluated following administration of drug at 7- or 21-day intervals. Accumulation was estimated from initial uptake, retention of unchanged drug in tumor tissues, tumor sensitivity, and growth rate. Data suggested that scheduling VCR every 7 days would be more effective than every 21 days, due to more rapid accumulation to cytotoxic levels. The effect of scheduling was examined in two rhabdomyosarcoma xenografts, where in vivo responses were similar to those predicted based upon drug uptake, retention, and growth characteristics for the tumors. Scheduling VCR at 7-day intervals was clearly superior to administration at 21-day intervals in these models. [Cancer Treat Rep 71:717-721, 1987]**

Vinca alkaloids form the basis for chemotherapy of many solid tumors of childhood. In the treatment of rhabdomyosarcoma (RMS), vincristine (VCR) is probably the most active single agent (1), whereas vinblastine (VLB) (which differs from VCR in substitution of a methyl group on the $N_a$ atom of the vindoline moiety) appears to be less active against xenografts derived from untreated RMS (2,3). This difference in efficacy between VCR and VLB appears to be a consequence of differences in the affinity of binding to tubulin (determined in membrane-free cytosols) and results in a more prolonged retention of VCR within tumor tissue (4). It is this prolonged retention in RMS tissue, with relatively rapid clearance of VCR from host (mouse) tissues, that leads to the high therapeutic activity of this vinca alkaloid in the RMS xenograft model (3). In this article, we have examined the accumulation of VCR in RMS xenografts and examined the effect of administration at 7- or 21-day intervals in two RMS xenografts with different VCR retentions and growth rates.

## MATERIALS AND METHODS

### Tumor Lines

HxRh12, HxRh18, and HxRh28 RMS xenografts have been described previously (2). HxRh18/VCR-3 was selected in vivo for resistance to VCR (5). Each xenograft was established from tumor material obtained prior to the patient's receiving either chemotherapy or radiotherapy. Each xenograft maintained its original histology and each demonstrated human LDH isoenzymes and human karyotype. Tumor pieces were implanted into the sc space of female CBA/CaJ mice that had been immune-deprived by thymectomy and total-body irradiation subsequent to priming with a single administration of cytarabine (200 mg/kg) (3).

### Growth Inhibition

Mice were given ip injections of various doses of VCR or an equivalent volume of vehicle (saline) when tumors had reached $\geq 1$ cm in diameter. Tumor diameters in both treated and control mice were determined at 7-day intervals using vernier calipers (2). Dose levels (mg/m$^2$) were calculated using the method of Freireich et al (6).

### Pharmacokinetics

[$^3$H]VCR (8 Ci/mmol) was purchased from Moravek Biochemicals (Brea, CA) and purified by high-performance liquid chromatography as described (7). Mice bearing bilateral tumors were given ip injections of [$^3$H]VCR (0.2 $\mu$Ci/g of body weight; specific activity

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between 5.5 and 550 mCi/mmol). For accumulation and retention studies tissues were sampled from 10 minutes to 72 hours after injection. To determine the relationship between dose of VCR and accumulation in tumor, tissues were excised and processed after 24 hours as described (3).

## RESULTS

In previous studies (4), we have noted that VCR, but not VLB, given as a single administration was very effective in causing complete regressions of HxRh12 xenografts. Thus, VCR appeared to have a greater effect than would be anticipated from a cell cycle phase-specific agent (8). The basis for the high therapeutic efficacy in RMS xenografts appeared to be due to prolonged retention of unchanged VCR in tumor tissue, with rapid elimination from non-neoplastic tissues of the host (mouse) (table 1) (3,5,9). Assuming, therefore, that a threshold concentration sufficient to achieve an antimitotic effect was exceeded, cytotoxicity occurred as cells entered mitosis. Hence, prolonged retention of VCR appeared sufficient to explain the cytotoxicity in tumors where cell cycle times may be on the order of 50–60 hours (10; and unpublished data). The relationship between retention of VCR and VLB and tumor sensitivity is shown in table 1. These data emphasize that drug retention in RMS is a major determinant of tumor sensitivity.

We were interested, therefore, in determining whether alteration in the frequency of scheduling of VCR at dose levels achievable in children had significant influence upon tumor response. Two schedules were considered (ie, 1.5 mg/m$^2$ given at intervals of either 7 or 21 days). These frequencies are used clinically and may be alternated during the course of therapy (11,12). Initially, the relationship between dose administered and drug accumulation in HxRh12 and HxRh18 tumors was examined. Tumor-bearing mice received a single injection of drug at doses between 1 and 100 mg/m$^2$ prior to the determination of the VCR concentration (fig 1). It was evident that over a 100-fold dose range drug accumulation was linear with no evidence of saturation, drug levels being related directly to drug dose. From these data and also from tumor growth rate and additional pharmacokinetic studies (3), the accumulation of VCR given every 7 or 21 days was calculated. Parameters used for these calculations are shown in table 2. Concentrations of drug designated cytostatic or cytotoxic were derived from considerable data in which tumor responses were determined subsequent to a single administration of drug. For each tumor line growth was inhibited for ≥ 7 days after 2.5 mg/m$^2$ of VCR and significant volume reduction (> 25%) occurred at 5.0 mg/m$^2$. To calculate drug accumulation, the initial drug concentration, loss rate, and tumor growth rate were used; further, it was assumed that no tumor growth occurred if drug achieved cytostatic concentration. Subsequent to bolus administration of VCR, retention of drug in RMS tissue is prolonged, whereas clearance from most non-

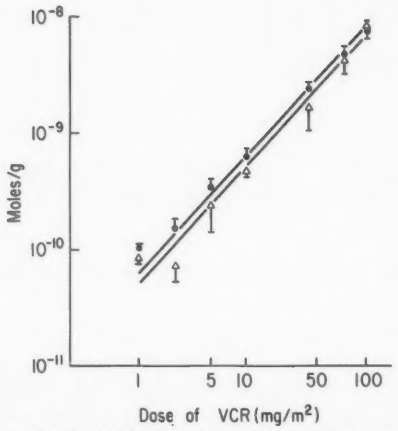


FIGURE 1.—Relationship between dose of VCR administered and drug accumulation in tumor. Tumor-bearing mice were given a single ip administration of VCR at dose levels between 1 and 100 mg/m$^2$. ● = HxRh12; △ = HxRh18. Tumors were excised 24 hrs later, and total drug concentration was determined. Results, mean ± SD ($n \geq 6$).

TABLE 1.—Retention of VCR*

| Tissue | t½α (hrs) | Tumor sensitivity† | Ref No. |
|---|---|---|---|
| Tumor (human RMS xenograft) | | | |
| Rh12 | 720 | ++++++ | 3 |
| Rh28 | 440 | +++++ | 3 |
| Rh18 | 240 | +++ | 3 |
| Rh18/VCR-3‡ | 40 | ± | 8 |
| Non-neoplastic (mouse) | | | 9 |
| Plasma | 0.37 | | |
| Small intestine | 1.67 | | |
| Liver | 1.23 | | |
| Kidney | 1.60 | | |
| Skeletal muscle | 9.60 | | |

*Clearance of total drug from tissue.

†Tumor response grading system used is as follows: ++++++ = complete regression with no regrowth; +++++ = complete regression with regrowth; +++ = growth inhibition ≥ 3 × $T_{D2}$; and ± = transient response, with inhibition < $T_{D2}$ (where $T_{D2}$ is mean tumor volume doubling time).

‡Subline of Rh18 selected for resistance to VCR in vivo (5).

TABLE 2.—Parameters used to calculate retention of VCR in xenografts of childhood RMS

| Tumor | $T_{D2}$ (days) | t½α (hrs) | Cytostatic dose (mg/m$^2$)* | Cytotoxic dose (mg/m$^2$)† |
|---|---|---|---|---|
| Rh12 | 14 | 720 | 2.5 | 5.0 |
| Rh18 | 10.3 | 240 | 2.5 | 5.0 |

*Determined by growth inhibition experiments following a single administration of VCR. Inhibition of growth ≥ 7 days.

†Minimum dose (single administration) causing significant reduction in tumor volume (≥ 25%).

neoplastic tissues is rapid (3); the concentration of VCR in sensitive tumor thus remains relatively constant. For example, administration of VCR at 2.5 mg/m$^2$ (cytostatic) gives a prolonged level in tumor approximately $1.5 \times 10^{-10}$ mols/g or $3 \times 10^{-7}$ M in cell water (determined to be approximately 50% of tumor mass).

Within the obvious constraints of such assumptions, drug accumulation was calculated for VCR administration given at 7- or 21-day intervals. Figure 2 shows calculated drug accumulation in HxRh12 and HxRh18 xenografts. In the HxRh12 xenograft, a cytotoxic concentration ($6 \times 10^{-7}$ M in cell water) would be achieved by Week 5 (1.5 mg/m$^2$ every 7 days), whereas concentration in the tumor after the third dose given every 21 days would have barely reached cytostatic levels. For HxRh18 tumors, which have a greater rate of drug loss than HxRh12 ($t_{1/2\alpha}$ = 240 hours), VCR levels approached cytostatic levels only over several weeks when drug was given every 7 days, but not on the less frequent schedule. Also shown in figure 2 is the accumulation of VCR projected for an every-7-day schedule at a dose of 5.0 mg/m$^2$ in HxRh18 tumors. In this case cytotoxic concentrations would be achieved by the second and subsequent administration.

To test the validity of these calculations, the efficacy of administration at different frequency and dosage was examined in mice bearing HxRh12 and HxRh18 xenografts. VCR was administered at 1.5 mg/m$^2$ given either at 7- or 21-day intervals. For HxRh12 tumors, responses (complete regressions) were assessed at Week 7. As shown in table 3, both schedule and dose dependency were observed in HxRh12 xenografts. At 1.5 mg/m$^2$, administration every 7 days caused complete regressions in seven of 12 tumors, with partial regressions (> 50% volume reduction) in five of 12 tumors. In contrast,

TABLE 3.—Schedule dependency for VCR in HxRh12 xenografts

| Dose (mg/m$^2$) | Schedule (days) | Complete regressions (%) | Total tumors |
|---|---|---|---|
| 1.16 | Every 7 | 4 (28) | 14 |
| 1.5 | Every 21 | 0 (0) | 14 |
| 1.5 | Every 7 | 7 (58) | 12 |
| 2.32 | Every 7 | 14 (100) | 14 |

there were no complete regressions and only two of 14 partial regressions when VCR was administered every 3 weeks at the same dosage. Data also show that the response rate to VCR (every 7 days) was dose-dependent in this tumor line. Only four of 14 tumors regressed fully at 1.16 mg/m$^2$, whereas all tumors regressed at 2.3 mg/m$^2$.

For HxRh18 tumors VCR at 1.5 mg/m$^2$ every 7 days did retard tumor growth slightly but did not cause regression (fig 3), whereas the calculated data suggested that growth would not be inhibited. At 5.0 mg/m$^2$ every 7 days (three doses), the mean tumor regression was ~70% for two groups comprising 14 tumors each. Clearly, at this dose cytotoxic concentrations were achieved as predicted (fig 2). Administration of 1.5 mg/m$^2$ every 21 days was not examined, however, because even after a single administration of 2.5 mg/m$^2$ tumors had progressed (2.7-fold increase in volume) by Day 21 (fig 3).

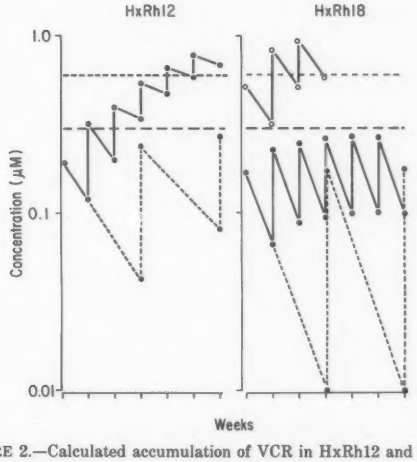


FIGURE 2.—Calculated accumulation of VCR in HxRh12 and HxRh18 tumors after administration at intervals of 7 (——) or 21 (-----) days at a dose level of 1.5 mg/m$^2$ or in HxRh18 at 7-day intervals at a dose of 5.0 mg/m$^2$ (○).

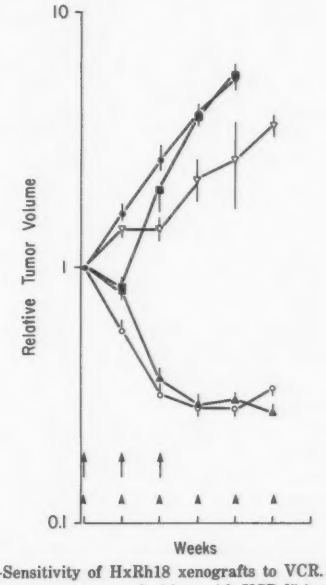


FIGURE 3.—Sensitivity of HxRh18 xenografts to VCR. Mice bearing bilateral tumors were treated either with VCR [5.0 mg/m$^2$ every 7 days for 3 (↑) courses (○, ▲); 1.5 mg/m$^2$ every 7 days for 6 (↷) courses (▽)], saline (●), or VCR at 2.5 mg/m$^2$ given as a single administration (■). Data represent the mean tumor volume relative to that at the initiation of treatment ± SE for groups containing 12–14 tumors.

## DISCUSSION

Several studies have indicated that in tumors sensitive to vinca alkaloids there is a prolonged retention of drug (3,13). In the HxRh12 xenograft, prolonged retention of VCR but not VLB determines the differential response to these two alkaloids (3). Similarly, short exposure of HL60/Cl cells to VCR and VLB revealed considerable differences in potency, which correlated with differential retention after removal of drug from the medium (14). Under conditions in vivo, vinca alkaloids are rapidly eliminated from plasma (15), and in the mouse, elimination of VCR or VLB, from most nonneoplastic tissues, paralleled elimination from plasma. The mechanism causing prolonged retention of VCR in xenografts of RMS remains to be elucidated, but there is a good correlation between drug retention and affinity of vinca binding to tubulin in membrane-free extracts from tumors, determined by Scatchard analysis (4).

In this study, we have attempted to model accumulation of VCR based on data previously derived using RMS xenografts. Two schedules were considered (every 7 or 21 days), since these have been used in treatment protocols for children with RMS (11,12) (E. Etcubanas, unpublished data). Initially, we determined that drug accumulation in tumors was directly related to the dose administered (fig 1), thus allowing modeling for a series of drug dose levels. Further, since accumulation was linear over a 100-fold dose range, it was unlikely that sequential administration of low doses of VCR would saturate intracellular binding capacity. Cytostatic and cytotoxic concentrations (in cell water) were estimated at $3 \times 10^{-7}$ M and $6 \times 10^{-7}$ M, respectively, from extensive studies in which tumor responses were measured following a single administration of VCR. The term cytostatic implies only that tumor growth is inhibited, which of course may reflect cell kill. The single parameter that correlated strongly with tumor response was retention of unchanged VCR in tumor tissue; thus, two tumors were chosen based upon differences in their retention of VCR. In HxRh12 and HxRh18, the half-life for retention ($t_{1/2}\alpha$) was determined to be 720 and 240 hours, respectively (3). A second parameter, rate of tumor growth, was included since tumor growth would cause a decrease in drug concentration (ie, dilution effect). Finally, the dose level of 1.5 mg/m$^2$ was chosen, since this is similar to that used in clinical treatment of RMS. Based upon these determinations and assumptions, the accumulation of VCR given at 7- and 21-day intervals was examined in each tumor. In each case more frequent scheduling would be expected to result in greater accumulation of VCR in tumor. However, in the HxRh18 xenograft, the least sensitive of the lines, it was calculated that at this dose neither schedule would be inhibitory to tumor growth. However, a higher dose (5.0 mg/m$^2$) given at 7-day intervals for three doses would be predicted to achieve cytotoxic concentration.

The effect of scheduling and dose was examined in mice bearing HxRh12 xenografts. For this study clinical criteria were used (complete and partial responses), measured 7 weeks after initiation of therapy. Thus, mice received six doses (every 7 days) compared with only three doses where VCR was given every 21 days. Further, it could be argued that measurement of outcome 1 week subsequent to the final dose did not allow measurement of the final effect of chemotherapy. However, we were attempting to use clinical criteria to assess response, and this is often evaluated at 6–9 weeks (11,16). Quite clearly, administration at 7-day intervals was superior to the less frequent schedule in HxRh12 tumors. Further, the responses were dose-dependent (table 2). For HxRh18 tumors, VCR (1.5 mg/m$^2$) administered at 7-day intervals did inhibit tumor growth slightly. This is in fair agreement with response predicted from drug accumulation calculations. When high-dose VCR (5.0 mg/m$^2$) was given for three doses at 7-day intervals, mean volume regression was approximately 70% (28 tumors), as predicted (fig 3). The effect of scheduling VCR at 21-day intervals was not examined, since previous experiments showed that 3 weeks after a single dose of 2.5 mg/m$^2$ the mean tumor volume had increased 2.7-fold (fig 3). Further, HxRh18 tumors had regrown to the original size in 3 weeks following a single administration of VCR (5.0 mg/m$^2$; data not shown).

These data support the contention that prolonged retention of VCR is of importance in determining tumor response, at least in RMS xenografts, and suggest that more frequent administration would be superior to 21-day intervals for VCR. Evaluation in two models of RMS confirms the superiority of administration at 7-day intervals. These data may have value in designing optimal clinical protocols for RMS using VCR. In maintenance protocols where VCR may be administered every 21 days, it may be more efficacious to administer "blocks" of VCR every 7 days periodically during maintenance. Such scheduling should not be precluded by excessive neurotoxicity. As with other agents, these data support the contention that efficacy is related to dose intensity (17).

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# Platinum Analogs in Recurrent and Advanced Head and Neck Cancer: A Southwest Oncology Group and Wayne State University Study[1,2,3]

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**Cisplatin combinations are active in patients with epidermoid cancer of the head and neck. Because of the incidence of dose-limiting toxicity and the need for hospitalization for iv hydration and mannitol diuresis, the search for active analog(s) with less of such toxicity has been intensive. In a limited institution study of Wayne State University and the Southwest Oncology Group, a clinical trial of carboplatin (CBDCA) and iproplatin (CHIP) in patients with recurrent head and neck cancer was carried out. Sixty-four patients were entered and 63 were evaluated, 29 receiving CBDCA and 34 receiving CHIP therapy. These patients were stratified according to important prognostic factors. The response rate to CBDCA was 24% (seven responses among 29 patients; three complete responses and four partial responses), and to CHIP was 12% (four responses among 34 patients; one complete response and three partial responses). Both drugs were administered without prior hydration or mannitol diuresis on an outpatient basis. The major side effect was myelosuppression, which was reversible but cumulative and dose-limiting. Less severe vomiting occurred as compared to the incidence of this toxicity with cisplatin and no significant renal or hearing loss occurred. It was concluded that further evaluation of these agents with other active drug(s) in patients with head and neck cancer is warranted. [Cancer Treat Rep 71:723-726, 1987]**

Cisplatin is one of the most active single agents in epidermoid carcinoma of the head and neck. In combination with other agents, especially 5-fluorouracil infusion, it produced a significant overall response rate and a clinically complete response in previously untreated patients with locally advanced cancer and in patients with recurrent or metastatic disease (1). A major dose-limiting toxic effect of cisplatin is peripheral neuropathy, which may occur after $> 600$ mg/m$^2$ has been administered. Other undesirable side effects of cisplatin include nausea and vomiting, renal impairment, and hearing loss (1-5).

Two platinum analogs, carboplatin (CBDCA) and iproplatin (CHIP), were found to be active in most cisplatin-sensitive animal tumors and certain human malignancies with fewer of the undesirable cisplatin toxic effects (6-17).

This is a report of a phase II limited institution clinical trial with CBDCA or CHIP in patients with recurrent and/or metastatic epidermoid cancer of the head and neck not previously treated palliatively with chemotherapy. This study was conducted by Wayne State University (WSU), Detroit, MI, as part of the National Cancer Institute phase II and III contract, and the Southwest Oncology Group (SWOG).

## MATERIALS AND METHODS

Patients with histologically proven recurrent and/or metastatic epidermoid cancer of the head and neck were included in this study. All patients had objectively measurable lesion(s) and had had no previous chemotherapy for recurrent disease. Patients with previous adjuvant chemotherapy 6 months before entrance in this trial were accepted into this study. All patients had good renal function, with creatinine clearance of at least 50 ml/minute, wbc count $\geq 4000$/mm$^3$, and platelet count $\geq 100{,}000$/mm$^3$. All patients signed informed consent forms before registration in the study.

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[3] Presented in part at the annual meeting of the American Society of Clinical Oncology, 1985.

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### Drug Dosage

Chemotherapy was administered without prior hydration or mannitol diuresis. CBDCA (400 mg/m$^2$) and CHIP (270 mg/m$^2$) were given iv over a 30-minute infusion on Day 1 and every 4 weeks. Doses were escalated or de-escalated according to the degree of myelosuppression. If no myelosuppression occurred after the first dose of chemotherapy, the drugs were increased only once (CBDCA to 440 mg/m$^2$; CHIP to 300 mg/m$^2$).

Chemotherapy was continued if patients achieved complete or partial response. Patients were removed from the study and followed for at least 4-6 weeks for toxicity evaluation if they had (*a*) stable disease after two courses, (*b*) disease progression after two courses, or (*c*) disease progression after the first dose with moderate to severe toxicity. All patients who received at least one course of chemotherapy were included in the analysis for response and toxicity.

### Stratification

This study was designed to test the activity and the incidence of side effects of two platinum analogs. The two phase II pilots were activated at the same time and patients were stratified according to performance status, previous radiotherapy, previous adjuvant chemotherapy with cisplatin combination, and extent of disease (locoregional disease vs systemic visceral metastasis). Patient assignment to treatment arms was done by the SWOG Statistical Office.

### Criteria of Response

Complete response (CR) was defined as the disappearance of all malignant lesion(s), without the appearance of new lesion(s). Partial response (PR) was defined as a $\geq 50\%$ decrease in the sum of the products of the two maximum diameters of the measurable and evaluable lesions, without the appearance of new lesion(s). Stable disease was defined as a response less than PR or increasing disease less than disease progression, without the appearance of new lesion(s). Disease progression was defined as a $> 25\%$ increase in the sum of the products of the two maximum diameters of the measurable lesion(s) and/or the appearance of new lesion(s) in responding or stable patients.

## RESULTS

Sixty-four patients were entered in this clinical trial: 30 patients received CBDCA and 34 patients received CHIP. Patients were evaluated for response and drug-induced toxicity. One patient died from non-treatment-related cardiac arrest 4 hours after receiving his first dose of CBDCA. Table 1 shows that patient characteristics, sex, race, age, performance status, and incidence of previous therapy were equally distributed between the two groups. All previous adjuvant chemotherapy contained cisplatin combinations.

TABLE 1.—Patient characteristics*

| Characteristic | CBDCA | CHIP |
|---|---|---|
| Sex | | |
| Male | 22 (76) | 26 (76) |
| Female | 7 (24) | 8 (24) |
| Race | | |
| White | 23 (79) | 23 (68) |
| Black | 6 (21) | 9 (26) |
| Other | | 2 (6) |
| Age (yrs) | | |
| Mean | 60 | 57 |
| Median | 58 | 56 |
| Range | 40-80 | 35-82 |
| Performance status | | |
| 0-1 | 19 (67) | 26 (76) |
| 2 | 9 (31) | 8 (24) |
| 3 | 1 (2) | |
| Previous therapy | | |
| Surgery | 25 (86) | 26 (76) |
| Radiotherapy | 23 (79) | 24 (71) |
| Chemotherapy | 8 (28) | 5 (15) |
| Primary site | | |
| Oropharynx | 9 (31) | 9 (36) |
| Oral cavity | 7 (24) | 7 (21) |
| Larynx | 9 (31) | 12 (35) |
| Pharynx | 3 (10) | 3 (9) |
| Unknown | 1 (3) | 3 (9) |
| Measurable lesions | | |
| Node/neck | 17 (59) | 23 (68) |
| Local | 7 (24) | 9 (26) |
| Lung | 9 (31) | 4 (12) |
| Liver | 2 (7) | 0 (0) |
| Bone | 0 (0) | 2 (6) |

*Unless otherwise specified, values = No. of patients (%).

### Response

Three patients had CR and four had PR after CBDCA treatments (24%), while four of 34 patients (12%) responded after CHIP treatments (one CR and three PRs). Stable disease occurred in 45% of the patients receiving CBDCA and in 50% receiving CHIP. All CRs occurred in patients with local and/or regional disease in the neck. PRs occurred in local (two), regional neck node (two), and lung (four) lesions. Responses were reported from all three institutions (three responses among 20 patients from Ohio State; three among 18 from San Antonio; and five among 25 from WSU). All responses occurred in patients with no previous adjuvant chemotherapy.

### Drug Toxicity

Myelosuppression was the major toxic effect caused by both agents. Tables 2 and 3 show the incidence and degree of drug-induced toxicity with CBDCA and CHIP, respectively. All toxic effects were reversible and no deaths occurred due to either agent. The incidence and severity of leukopenia, thrombocytopenia, and anemia were similar between the two groups. These side effects

TABLE 2.—Toxicity of CBDCA*

| Toxic effect | Degree of toxicity | | | | | No. with toxic effects (%) |
|---|---|---|---|---|---|---|
| | None | Mild | Moderate | Severe | Life-threatening | |
| Hematologic | | | | | | |
| Leukopenia† | 9 | 6 | 9 | 4 | 1 | 20 (69) |
| Thrombocytopenia‡ | 13 | 4 | 4 | 2 | 6 | 16 (55) |
| Anemia | 10 | 4 | 7 | 7 | 1 | 19 (66) |
| Gastrointestinal | | | | | | |
| Nausea and vomiting | 17 | 7 | 4 | 1 | 0 | 12 (41) |
| Diarrhea | 27 | 1 | 1 | 0 | 0 | 2 (7) |
| Other | | | | | | |
| Hearing loss | 28 | 1 | 0 | 0 | 0 | 1 (3) |
| Vertigo | 28 | 1 | 0 | 0 | 0 | 1 (3) |
| Alopecia | 28 | 1 | 0 | 0 | 0 | 1 (3) |

*Values = No. of patients.
†Mild = 3000–3999 cells/$mm^3$; moderate = 2000–2999 cells/$mm^3$; severe = 1000–1999 cells/$mm^3$; and life-threatening = < 1000 cells/$mm^3$.
‡Mild = 75,000–99,999 cells/$mm^3$; moderate = 50,000–74,999 cells/$mm^3$; severe = 25,000–49,999 cells/$mm^3$; and life-threatening = < 25,000 cells/$mm^3$.

were cumulative, with increase in severity and duration with subsequent doses. Many patients needed blood and/or platelet transfusion while receiving therapy.

Nine patients receiving CBDCA had no myelosuppression after the first dose, and the second dose was increased to 440 mg/$m^2$, while four patients with response or stable disease needed decrease of their second dose of CBDCA due to moderately severe or greater myelosuppression. Eight patients receiving CHIP had dose escalation following the first course of treatment, while four patients needed de-escalation of the doses of CHIP due to significant myelosuppression.

### Duration of Response

Duration range for the responders receiving CBDCA was 8–72 weeks, with a median of 12 weeks, and for CHIP was 8–18 weeks, with a median of 8 weeks.

### Survival

With median follow-up of 18 months, four patients on the CBDCA arm and seven patients on the CHIP arm are still alive, while the rest of the patients have either died (47 patients) or been lost to follow-up (five).

Survival time range of patients treated with CBDCA was 2–132+ weeks, with a median of 26 weeks. Survival time range of patients receiving CHIP therapy was 2–104+ weeks, with a median of 20 weeks. The overall survival of the responding patients ranged from 14 to 75 weeks, with a median of 34 weeks. All of the known deaths occurred due to progression of the malignant disease or in progressing patients.

## DISCUSSION

Cisplatin combinations seem to be the most active in patients with previously untreated locally advanced

TABLE 3.—Toxicity of CHIP*

| Toxic effect | Degree of toxicity | | | | | No. with toxic effects (%) |
|---|---|---|---|---|---|---|
| | None | Mild | Moderate | Severe | Life-threatening | |
| Hematologic | | | | | | |
| Leukopenia† | 15 | 7 | 7 | 4 | 1 | 19 (56) |
| Thrombocytopenia‡ | 14 | 5 | 4 | 8 | 4 | 20 (59) |
| Anemia | 12 | 9 | 11 | 1 | 1 | 22 (65) |
| Gastrointestinal | | | | | | |
| Nausea and vomiting | 13 | 4 | 11 | 4 | 2 | 21 (62) |
| Diarrhea | 32 | 1 | 1 | 0 | 0 | 2 (6) |
| Other | | | | | | |
| Renal | 32 | 2 | 0 | 0 | 0 | 2 (6) |
| Neuropathy | 32 | 2 | 0 | 0 | 0 | 2 (6) |

*Values = No. of patients.
†*See* definitions in table 2.
‡*See* definitions in table 2.

head and neck cancer, producing an overall response rate ≤ 90% and a clinical CR rate ≤ 50% after two to three courses (1).

Cisplatin combinations are the most active chemotherapies in patients with recurrent and metastatic epidermoid cancer of the head and neck (1). The dose-limiting peripheral neuropathy produced by cisplatin and other serious side effects, especially vomiting and renal impairment, create problems in long-time administration of this agent and compliance of these patients. This led to the search for second-generation platinum compounds which might be free of some of these serious side effects. In the preclinical testing and early clinical trials, both platinum analogs CBDCA and CHIP retained the antitumor activity of cisplatin with less toxicity (6–17).

The overall response rates to CBDCA and CHIP in this study were 24% and 12%, respectively. These response rates are similar to the response rate reported produced by single-agent cisplatin (1,18–20).

As predicted from preclinical studies and early human trials, the dose-limiting toxic effect for both CBDCA and CHIP is myelosuppression. The incidence range of leukopenia, thrombocytopenia, and anemia is 55%–69%.

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# Combination Chemotherapy With a New Folate Analog: Activity of 10-Ethyl-10-deaza-aminopterin Compared to Methotrexate With 5-Fluorouracil and Alkylating Agents Against Advanced Metastatic Disease in Murine Tumor Models[1,2]

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**A new folate analog, 10-ethyl-10-deaza-aminopterin (10EDAM), was compared to methotrexate (MTX) in combination with 5-fluorouracil (5-FU) and/or alkylating agents against three models of advanced metastatic (E0771 mammary adenocarcinoma and T241 fibrosarcoma) or disseminated (L1210 leukemia) disease in $BD2F_1$ mice. Of all the agents examined on a schedule of every 3 days × 3, 10EDAM and cyclophosphamide (CPA) were the most active overall against the tumor models employed. Against the E0771 tumor, antitumor potency was in the descending order of CPA, 10EDAM, cisplatin (*Cis* Pt), melphalan (L-PAM), MTX, and 5-FU. Similar ranking in relative potency was also derived with the T241 tumor. Against both of these models, MTX and 5-FU were only minimally active. Against the L1210 leukemia, 10EDAM was the most active agent followed in descending order by MTX, CPA, 5-FU, L-PAM, and *Cis* Pt. All two-drug combinations required attenuation to one-half the LD10 dosage for each. The results show that 10EDAM with CPA was the most effective combination employed in all three tumor models. Therapeutic activity was far greater than that obtained with each agent alone, with a substantial number of long-term survivors. 10EDAM was also highly active in combination with L-PAM and *Cis* Pt against the E0771 tumor and with *Cis* Pt against the L1210 leukemia. Some increase in therapeutic activity beyond that obtained with each agent alone, but to a substantially lesser extent, was documented for MTX and each of the three alkylating agents against the L1210 tumor. MTX with 5-FU was not an effective combination, but against the E0771 tumor gave therapeutic effects beyond that obtained with each agent alone when 5-FU was given 7 hours after MTX. Similar effects were seen with 10EDAM and 5-FU on these schedules, but 10EDAM alone was more effective than MTX and 5-FU given together against this tumor. A combination of either antifolate with 5-FU and CPA was ineffective against all three tumor models, in part because of the further attenuation of dosage required in this mouse strain and the minimal overall activity of 5-FU in these models. Results documented with 10EDAM and CPA and, perhaps, other alkylating agents are interpreted as indicative of therapeutic synergism between these agents. These results appear to suggest substantial potential for 10EDAM in clinically employed combination therapy currently including MTX. [Cancer Treat Rep 71:727-732, 1987]**

Our earlier results (1) with the new folate analog 10-ethyl-10-deaza-aminopterin (10EDAM) documented marked improvement in therapeutic efficacy of this analog compared to methotrexate (MTX) against a variety of ascitic and solid murine tumor models. A large differential in antitumor activity favoring 10EDAM was also obtained (2) against three human tumors, lung carcinoma, mammary carcinoma, and colon carcinoma, xenografted into nude mice. In these studies a substantial number of complete regressions were documented (2) with 10EDAM while none were obtained with MTX. This apparent large improvement in relative therapeutic efficacy of 10EDAM over MTX in these models was predicted by the results (3) of our biochemical and pharmacologic studies of this agent. 10EDAM emerged from a program of synthetic design which exploited (4-6) evidence showing that the N10 position of the folate molecule was specified for membrane transport

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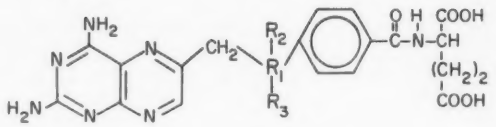
and polyglutamylation, but was relatively unspecified for inhibition of the intracellular target dihydrofolate reductase. This meant that a number of structural modifications at this site altered the extent of membrane transport and polyglutamylation but had no effect on inhibition of the target enzyme. Also, the extent to which this position on this molecule was specified for membrane transport and polyglutamylation varied for tumor compared to normal proliferative tissues. Of the group of new structures modified at the N10 position subsequently examined (3), 10EDAM showed the largest differential in membrane transport and polyglutamylation between various tumors versus normal proliferative tissues which more highly favored a preferential accumulation of 10EDAM as a polyglutamate in these tumors compared to other analogs in this class and MTX.

In a further extension of this developmental program, we have been examining the efficacy of 10EDAM vis-à-vis MTX in combination with other cytotoxic agents. These included studies of combinations incorporating MTX that are now used clinically. We now report on our studies of these antifolates in combination with 5-fluorouracil (5-FU) and cyclophosphamide (CPA) against metastatic murine mammary adenocarcinoma E0771 and two additional models of advanced disseminated disease (metastatic T241 fibrosarcoma and advanced L1210 leukemia). In view of the interesting results obtained with the combination of CPA and 10EDAM, additional studies were also carried out in these models with two other alkylating agents, melphalan (L-PAM) and cisplatin (*Cis* Pt). These results are reported below.

## MATERIALS AND METHODS

Many of the experimental procedures employed during these studies have already been described in detail in earlier reports (1,2) from our laboratory. All of the folate compounds employed were prepared as the sodium salt in neutral aqueous solution. The synthesis of 10EDAM has been reported elsewhere (7). Samples of MTX used were provided by the Drug Procurement and Synthesis Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD. All compounds employed were analyzed (7) and found to be > 95% pure. The structures of both folate analogs used in these studies are shown in figure 1. The E0771, T241, and L1210 neoplasms were maintained in $BD2F_1$ mice by sc or ip transplantation. For therapeutic experiments, $10^5$ to $10^6$ tumor cells in suspension were transplanted im or iv (E0771 or T241) or ip (L1210) into female mice. Mice receiving implants of E0771 or T241 cells invariably died with extensive metastatic disease in the lung. Mice implanted with L1210 cells displayed evidence of extensive organomegaly at the time of death. Therapy was begun 2, 3, 4, or 6 days following transplantation using a schedule of once per day every 3 or 4 days, for a total of three injections (every 3 days × 3 or every 4 days × 3). Tolerances to these agents on this schedule were established by a series of preliminary experiments in non-tumor-bearing



| $R_1$ | $R_2$ | $R_3$ | Analog |
|---|---|---|---|
| N | $CH_3$ | — | methotrexate |
| C | H | H | 10-deazaaminopterin |
| C | $C_2H_5$ | H | 10-ethyl-10-deaza-aminopterin |

FIGURE 1.—Structural formulas for folate analogs.

animals. Dosing during the studies described here was at or near the LD10 dosage or at a defined fraction thereof. In some experiments 5-FU was administered with a 7-hour delay after MTX or 10EDAM. Antitumor effects were expressed as percent increase in lifespan (ILS) based on the median survival time (MST) of treated versus control (untreated) groups. Toxicity was monitored by weight loss and drug-induced deaths in animals with no evidence of tumor growth. Drug tolerances in tumor-bearing and non-tumor-bearing animals were similar. Analysis for statistical significance was carried out using the Chi-square test as described by Zar (8).

## RESULTS

Our initial studies with the E0771 mammary adenocarcinoma focused on advanced metastatic disease and its therapy with single agents following im transplantation of this tumor in the hind leg of $BD2F_1$ mice. After a delay of 12 days, one-half of the animals implanted with $10^5$ tumor cells were randomly selected for tumor excision by surgery and both surgery and nonsurgery groups were treated 1 day later with either MTX, 10EDAM, or 5-FU at the approximate LD10 dosage of each on a schedule of every 4 days × 3. The results of this experiment are shown in table 1. In the group with tumors intact, animals receiving no chemotherapy died by 17–19 days, invariably with multiple tumors in each lung. Treatment of mice with either of the three agents had little or no effect (data not shown) on the original tumor. Also, treatment with MTX had no significant impact on survival time. Treatment with 5-FU had a small effect, but 10EDAM had a pronounced effect on survival time (33% vs 83%; $P < 0.05$).

Only one of the 14 untreated animals with tumors excised was a long-term survivor. Treatment with either MTX or 5-FU had little effect (< 25% ILS) on the median survival time but some impact on the number of long-term survivors. Within the group treated with 10EDAM, all but one of the animals were long-term survivors and the MST was significantly better than either of the other treated groups ($P < 0.001$).

TABLE 1.—Antitumor effects of MTX, 10EDAM, and 5-FU against metastatic E0771 mammary adenocarcinoma

| | Tumor intact | | Tumor excised† | | |
|---|---|---|---|---|---|
| LD10 dosage* agent | MST ± SE (days) | ILS (%) | MST (days) | ILS (%) | 60-day survivors/ total treated‡ |
| — | 18 ± 2.8 | | 37.5 | | 1/14 |
| MTX | 19 ± 4.4 | 5 | 43.5 | 16 | 5/14 |
| 5-FU | 24 ± 5.1 | 33 | 39.5 | 5 | 3/14 |
| 10EDAM | 33 ± 6.5 | 83 | > 60 | > 60 | 13/14 |

*MTX (70 mg/kg), 5-FU (66 mg/kg), and 10EDAM (140 mg/kg) given once every 4 days for a total of 3 doses starting 12 days after tumor implant ($10^5$ cells) im. ($n = 2$, 7 mice/group).

†Tumor excised on day prior to therapy.

‡Animals were tumor-free at autopsy.

In an effort to derive a more suitable model for metastatic disease for use in these experiments, we have employed iv administration of suspensions of E0771 cells. Inoculation of $10^6$ tumor cells by this route results in metastatic disease of the lung and death of these animals in 11–12 days. The progression of disease in these animals was very similar to that seen for im administered tumor, although somewhat more rapid in onset. Since the agents under study show differing potencies against this tumor, we carried out a preliminary experiment examining the effect of the approximate LD10 dosage of these agents on metastatic disease following varying periods of delay prior to therapy. The objective of these studies was to obtain conditions which would adequately minimize the therapeutic effect of individual agents, thus allowing their evaluation in combination in a reasonable fashion. The results of this experiment are given in table 2. Following a 2-day delay, treatment with any of the drugs on an every 3 days × 3 schedule produced a significant increase in MST as compared to the untreated controls ($P < 0.001$). However, treatment with 5-FU or MTX resulted only in a modest increase in survival time and no long-term survivors, whereas 10EDAM was partially curative and CPA was completely curative. Following a delay of 4 days, the therapeutic effect of all four agents was reduced from that seen following a 2-day delay, but CPA and 10EDAM were still partially curative. Following a delay of 6 days, when tumor-bearing animals had already lost weight and showed other signs of advanced disease (all animals killed on Day 6 showed extensive metastatic disease in the lungs), 5-FU was ineffective ($P > 0.10$), MTX had a small effect ($P < 0.01$), but CPA and 10EDAM had an ILS approximately twofold over controls ($P < 0.01$). No long-term survivors were obtained following a 6-day delay of therapy with any of these agents.

Starting therapy 6 days after iv implantation of $10^6$ E0771 cells, we have compared MTX and 10EDAM in combination with either 5-FU or CPA. These combinations were compared at ¾ and ½ of the LD10 dosage for each agent on a schedule of every 3 days × 3. Combinations employing ¾ of the LD10 dosage of these agents were toxic (20%–30% of deaths were drug-related). However, as seen in table 3, mice tolerated ½ of the LD10 dosage of these agents in combination. MTX with 5-FU at these dosages resulted in an MST and an ILS slightly greater than MTX alone at its LD10 dosage (table 2). A similar result was obtained with MTX plus CPA and 10EDAM plus 5-FU. A 7-hour delay in administration of 5-FU with MTX or 10EDAM resulted in an enhanced therapeutic effect (table 3) for both combinations ($P < 0.025$). However, with either simultaneous or sequential administration, combinations employing MTX were not as active as 10EDAM alone ($P < 0.001$). The combination of 10EDAM with CPA was highly effective and cured most of the mice in this treated group and was significantly better than any other combination or single-drug treatment ($P < 0.001$). We also evaluated triple combinations against this model including either MTX or 10EDAM with both 5-FU and CPA. Both combinations were toxic at ½ the LD10 dosage of each agent. Attenuation to ⅛ of the individual LD10 dosages was tolerated but antitumor activity was less than, or no more than equivalent to, that obtained with CPA or 10EDAM alone at their LD10 dosage ($P > 0.10$).

In view of the result obtained with CPA in combination with 10EDAM in this model, we examined combinations employing both antifolates with either one of two other alkylating agents, L-PAM or *Cis* Pt. These results are given in table 4. Following a 6-day delay, both of these agents at the approximate LD10 dosage exhibited considerable activity against this model which was somewhat comparable to 10EDAM. Similar to combinations employing CPA, both L-PAM and *Cis* Pt, with

TABLE 2.—Effect of single agents on iv administered E0771 mammary adenocarcinoma

| Days to therapy* | Agent† | MST ± SE (days) | ILS (%) | 45-day survivors/ total treated‡ |
|---|---|---|---|---|
| 2 | — | 12 ± 0.8 | | 0/20 |
| | 5-FU | 20 ± 1.3 | 67 | 0/14 |
| | CPA | > 45 | > 275 | 14/14 |
| | MTX | 22 ± 2.0 | 83 | 0/13§ |
| | 10EDAM | 28 | 133 | 6/14 |
| 4 | — | 12 ± 0.8 | | 0/20 |
| | 5-FU | 18 ± 1.3 | 50 | 0/14 |
| | CPA | > 45 | > 275 | 8/13§ |
| | MTX | 20.5 ± 1.9 | 71 | 0/14 |
| | 10EDAM | 27.5 | 129 | 4/14 |
| 6 | — | 11.5 ± 0.8 | | 0/20 |
| | 5-FU | 12 ± 0.8 | 4 | 0/14 |
| | CPA | 21.5 ± 2.3 | 87 | 0/14 |
| | MTX | 14 ± 1.4 | 22 | 0/14 |
| | 10EDAM | 24 ± 2.9 | 108 | 0/14 |

*Therapy was begun 2, 4, or 6 days after iv implantation of $10^6$ tumor cells.

†Approximate LD10 dosages of 5-FU (66 mg/kg), CPA (100 mg/kg), or 10EDAM (140 mg/kg) were given on a schedule of once every 3 days for a total of 3 doses ($n = 2$, 7–10 mice/group).

‡None of these animals showed evidence of tumor growth at the time of autopsy.

§1 toxic death (not utilized for calculations of MST).

TABLE 3.—Antitumor effects of MTX and 10EDAM in combination with 5-FU and/or CPA against iv administered E0771 mammary adenocarcinoma

| LD10 dosage* | Agent | MST ± SE (days) | ILS (%) | 45-day survivors/ total treated† |
|---|---|---|---|---|
| | — | 12 ± 0.9 | — | 0/21 |
| 3/4 | MTX, 5-FU | Toxic | | |
| | 10EDAM, 5-FU | Toxic | | |
| | MTX, CPA | Toxic | | |
| | 10EDAM, CPA | Toxic | | |
| 1/2 | MTX, 5-FU | 16 ± 2.2 | 33 | 0/14 |
| | 10EDAM, 5-FU | 23 ± 3.6 | 92 | 0/14 |
| | MTX, 5-FU‡ | 20 ± 3.9 | 67 | 0/14 |
| | 10EDAM, 5-FU‡ | 29 ± 4.2 | 142 | 0/14 |
| | MTX, CPA | 25 ± 4.2 | 108 | 0/14 |
| | 10EDAM, CPA | > 45 | > 275 | 9/14 |
| 1/3 | MTX, 5-FU, CPA | 23 ± 1.9 | 92 | 0/14 |
| | 10EDAM, 5-FU, CPA | 28.5 ± 4.0 | 138 | 0/14 |

*Therapy begun 6 days after transplanting $10^6$ tumor cells iv with 3/4, 1/2, or 1/3 of the LD10 dose of MTX (70 mg/kg), 10EDAM (140 mg/kg), 5-FU (66 mg/kg), or CPA (100 mg/kg) on a schedule of once/day every 3 days for a total of 3 doses ($n$= 2-3, 7 mice/group).

†Animals were tumor-free on Day 45.

‡5-FU was given 7 hrs after MTX or 10EDAM.

either antifolate, were toxic when given at 3/4 of the LD10 dosage for each agent. At 1/2 the LD10 dosage, combinations employing either alkylating agent with MTX resulted in therapeutic effects lower than that obtained with either alkylating agent alone at its LD10 dosage. In sharp contrast to these results, combinations employing either alkylating agent with 10EDAM showed marked therapeutic activity ($P < 0.001$). Lifespan was increased beyond that obtained with each agent alone (table 4) and a number of long-term survivors were obtained with each combination.

Experiments similar to those described above were also carried out with the T241 fibrosarcoma and the L1210 leukemia. The same model of metastatic disease as was developed with the E0771 tumor was developed for the T241 tumor. Therapy for this tumor was initiated ip 6 days following iv implant of $2 \times 10^5$ cells. At this time all of the animals exhibited metastatic disease in the lung. Therapy for the L1210 leukemia was begun sc 3 days after ip implant of $10^6$ cells, when the total tumor burden was 60-70 million cells and the animals exhibited pronounced organomegaly. The results of single-agent therapy of these tumor models at the LD10 dosage every 3 days × 3 are given in table 5. 5-FU, MTX, L-PAM, and *Cis* Pt were not significantly active ($P > 0.5$) against the T241 tumor. Both 10EDAM and CPA showed

TABLE 4.—Antitumor effects of MTX and 10EDAM in combination with L-PAM or *Cis* Pt against iv administered E0771 mammary adenocarcinoma

| LD10 dosage* | Agent | MST ± SE (days) | ILS (%) | 45-day survivors/ total treated† |
|---|---|---|---|---|
| | — | 12 ± 1.3 | | 0/14 |
| 1 | MTX | 14 ± 2.8 | 17 | 0/14 |
| | 10EDAM | 24 ± 3.5 | 100 | 0/13‡ |
| | L-PAM | 22 ± 2.9 | 83 | 0/14 |
| | *Cis* Pt | 22 ± 3.4 | 83 | 0/14 |
| 3/4 | MTX, L-PAM | Toxic | | |
| | 10EDAM, L-PAM | Toxic | | |
| | MTX, *Cis* Pt | Toxic | | |
| | 10EDAM, *Cis* Pt | Toxic | | |
| 1/2 | MTX, L-PAM | 19 ± 3.8 | 58 | 0/13‡ |
| | 10EDAM, L-PAM | 31.5 | 163 | 3/14 |
| | MTX, *Cis* Pt | 19.5 ± 3.5 | 63 | 0/14 |
| | 10EDAM, *Cis* Pt | 30 | 150 | 4/14 |

*Therapy begun ip 6 days after transplantation of $10^6$ tumor cells iv with the LD10 dosage or 3/4 or 1/2 of the LD10 dosage of MTX (70 mg/kg), 10EDAM (140 mg/kg), L-PAM (6 mg/kg), or *Cis* Pt (4 mg/kg) ($n$ = 2, 7 mice/group).

†Animals tumor-free on Day 45.

‡1 toxic death (not utilized for calculations of MST).

TABLE 5.—Antitumor effects of MTX, 10EDAM, 5-FU, or alkylating agents against metastatic T241 fibrosarcoma and advanced L1210 leukemia

| LD10 dosage* agent | T241 MST ± SE (days) | T241 ILS (%) | T241 45-day survivors/ total treated† | L1210 MST ± SE (days) | L1210 ILS (%) | L1210 45-day survivors/ total treated† |
|---|---|---|---|---|---|---|
| — | 14 ± 0.7 | — | 0/17 | 7 ± 0.3 | — | 0/15 |
| 5-FU | 18 ± 1.5 | 29 | 0/12 | 10 ± 1.0 | 43 | 0/17 |
| MTX | 18 ± 1.4 | 29 | 0/11 | 16 ± 1.8 | 129 | 0/13 |
| 10EDAM | 23 ± 1.6 | 64 | 0/11 | 19 ± 2.0 | 171 | 0/13 |
| CPA | 25 | 79 | 1/11 | 15 ± 1.5 | 114 | 0/11‡ |
| L-PAM | 17 ± 0.7 | 21 | 0/11 | 9 ± 1.1 | 29 | 0/10 |
| *Cis* Pt | 18 ± 0.6 | 29 | 0/11 | 9 ± 0.9 | 29 | 0/10 |

*MTX (70 mg/kg), 5-FU (66 mg/kg), 10EDAM (140 mg/kg), CPA (100 mg/kg), L-PAM (6 mg/kg), or *Cis* Pt (4 mg/kg). In T241, drugs administered ip starting 6 days after iv injection of $2 \times 10^5$ cells. In L1210, drugs administered sc starting 3 days after ip injections of $10^6$ cells ($n = 2$, 5–10 mice/group).

†Animals tumor-free on Day 45.

‡1 toxic death (not utilized for calculations of MST).

greater therapeutic effects ($P < 0.001$). Although CPA appeared to exhibit the greatest activity with one cure evident in this group, the difference between 10EDAM and CPA was not significant ($P > 0.50$). Thus, with the exception perhaps of CPA, this tumor was relatively less sensitive to these agents than the E0771 tumor. Against advanced L1210 leukemia, it can be seen (table 5) that all drugs except L-PAM and *Cis* Pt showed significant activity ($P < 0.001$). 10EDAM was the most active of the group of agents studied on this schedule of administration followed by MTX and CPA ($P < 0.001$ for 10EDAM compared with MTX). None of these agents were able to effect cures in this model of advanced disease.

Since combinations of these agents at ¾ of the LD10 dosages were already shown to be toxic in other experiments (tables 3 and 4), therapeutic efficacy of these combinations was compared at only ½ the LD10 dosage. These data are shown for both tumor models on the same schedule of administration (every 3 days × 3) in table 6. Against the T241 tumor, only the combination of 10EDAM with CPA resulted in substantial therapeutic activity ($P < 0.005$ when compared with control or LD10 dosage of CPA or 10EDAM). In addition to the marked ILS obtained compared to each agent alone, almost half of the animals treated with this combination appeared to be cured of their disease. Combinations employing MTX with 5-FU or alkylating agents and 10EDAM with either 5-FU, L-PAM, or *Cis* Pt were all significantly less effective than 10EDAM with CPA ($P < 0.01$). In contrast to the results obtained with the T241 tumor, all of the combinations employing two agents appeared to be effective against the L1210 leukemia. However, in combinations employing MTX with any of the alkylating agents, ILS observed did exceed that obtained with each agent alone. These increases for each combination compared to individual drugs alone

TABLE 6.—Antitumor effects of MTX and 10EDAM in combination with 5-FU and/or alkylating agents against metastatic T241 fibrosarcoma and advanced L1210 leukemia

| LD10 dosage* | Agent | T241 MST ± SE (days) | T241 ILS (%) | T241 45-day survivors/ total treated† | L1210 MST ± SE (days) | L1210 ILS (%) | L1210 45-day survivors/ total treated† |
|---|---|---|---|---|---|---|---|
| — | — | 14 ± 0.5 | — | 0/14 | 7 ± 0.3 | — | 0/14 |
| ½ | MTX, 5-FU | 18.5 ± 0.8 | 32 | 0/12 | 16 ± 0.8 | 129 | 0/10 |
| | MTX, CPA | 22 ± 1.6 | 57 | 0/16 | 18.5 ± 0.6 | 164 | 0/10 |
| | MTX, L-PAM | 19.5 ± 1.4 | 39 | 0/16 | 18 ± 1.1 | 157 | 0/10 |
| | MTX, *Cis* Pt | 18.5 ± 1.1 | 32 | 0/16 | 17 ± 0.4 | 143 | 0/10 |
| ½ | 10EDAM, 5-FU | 22 ± 1.6 | 57 | 0/12 | 19 ± 1.4 | 171 | 0/10 |
| | 10EDAM, CPA | 35 | 150 | 6/16 | > 35 | > 400 | 5/10 |
| | 10EDAM, L-PAM | 25 | 79 | 1/16 | 19.5 | 179 | 1/10 |
| | 10EDAM, *Cis* Pt | 23 ± 1.4 | 64 | 0/16 | 21 | 200 | 2/10 |
| ⅛ | MTX, 5-FU, CPA | 19 ± 1.0 | 36 | 0/10 | 17 ± 1.5 | 143 | 0/10 |
| | 10EDAM, 5-FU, CPA | 25 ± 3.9 | 79 | 0/10 | 19 ± 1.6 | 171 | 0/10 |

*For details of treatment, *see* footnote in table 5.

†Animals tumor-free on Day 45.

were not statistically significant ($P > 0.10$). In contrast, combinations of 10EDAM with all four agents gave greater therapeutic activity than the corresponding MTX combination ($P < 0.05$). ILS obtained with 10EDAM with CPA over that obtained with single agents alone was very substantial ($P > 0.001$) and a number of long-term survivors were obtained. Finally, as in the case of the E0771 tumor, we evaluated triple combinations of each antifolate with both 5-FU and CPA against T241 and L1210 (table 6). CPA was the most active of the three alkylating agents examined in these studies both as a single agent and in combination. It can be seen that at the required level of attenuation to ⅛ the LD10 dosage of each agent alone, the ILS obtained against either tumor was less than or equal to the result obtained with either antifolate plus CPA.

## DISCUSSION

These results extend earlier studies (1,2) with model murine systems and human tumor xenografts which documented appreciable therapeutic superiority of 10EDAM compared to MTX. Against advanced metastatic or disseminated disease in three murine models, 10EDAM again appeared to be clearly superior to MTX in the treatment schedule employed. Of all the agents examined, 10EDAM and CPA were the most active overall with the tumor models employed. Against the E0771 mammary adenocarcinoma, antitumor potency was in the descending order of CPA, 10EDAM, *Cis* Pt, L-PAM, MTX, and 5-FU. Similar ranking in relative potency was also derived with the T241 fibrosarcoma. Against both of these models, MTX and 5-FU were only minimally active. These results contrast somewhat with that obtained against advanced L1210 leukemia. 10EDAM was the most active agent followed in descending order by MTX, CPA, 5-FU, L-PAM, and *Cis* Pt. The results of these studies taken together with those reported earlier (1,2) from our laboratory strongly suggest that the therapeutic superiority of 10EDAM compared to MTX against these murine models is essentially schedule-independent. These antifolates have been compared in these models on a schedule of once per day × 5, once every other day × 5, and once every 3 or 4 days × 3 with similar relative efficacies.

The results also show that 10EDAM with CPA was the most effective combination of agents studied. This combination was highly curative against all three models of advanced disease. 10EDAM was also highly effective in combination with L-PAM and *Cis* Pt against the E0771 tumor and with *Cis* Pt against the L1210 tumor. Despite the dosage attenuation required, the level of therapeutic activity obtained in all these cases was considerably greater than that activity obtained with each agent alone at its LD10 dosage. We interpret these results, which show greater than additive therapeutic effects, as indicative of synergism between this new antifolate and these alkylating agents. Some evidence of synergism, but to a substantially lesser extent, is also documented for MTX and these three alkylating agents against the L1210 leukemia. We point out that similar findings in the case of MTX and CPA against the L1210 leukemia were reported earlier by others (9).

Our results also suggest that combinations incorporating 10EDAM or MTX with 5-FU and CPA (or probably other alkylating agents) may offer little in the way of increased antitumor effectiveness in other tumor models in which 5-FU shows only modest activity. This appears, to a great extent, to be due to the attenuation in dosage required to prevent toxicity of the three-drug combination. To what extent these results can be extrapolated to other rodent tumor models or to a clinical level will depend on the degree of attenuation in dosage required in these cases. It was of some interest to note, however, that the sequential administration of MTX or 10EDAM followed by 5-FU (table 3) did result in increased therapeutic activity against the E0771 tumor.

These results would appear to have some relevance to the utility of 10EDAM in the treatment of human cancer in the currently prevailing clinical setting. All three tumors employed were models of either advanced metastatic or disseminated disease not unlike that encountered clinically. Moreover, the three alkylating agents that were found to exhibit substantial therapeutic activity in combination with 10EDAM in these models are all currently employed in the treatment of patients with various neoplastic disorders. Our results would seem to suggest that there is substantial potential for 10EDAM in this clinical setting, particularly in those combinations including CPA.

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# Cardiotoxicity of 5-Fluorouracil[1]

**Carolyn Collins and Paul L. Weiden[2,3,*]**

**Three patients developed anterior substernal chest pain in association with ischemic electrocardiographic changes temporally related to continuous infusions of 5-fluorouracil (5-FU). Two patients developed myocardial infarctions and one died. Cardiac toxicity of 5-FU may be more likely when the drug is given by continuous infusion in the presence of pre-existing cardiac disease. The pattern of cardiac toxicity suggests cardiac ischemia most likely secondary to coronary artery vasospasm. Patients should not receive 5-FU by infusion if they have significant underlying coronary artery disease or if they develop anterior substernal chest pain while receiving the drug. [Cancer Treat Rep 71:733-736, 1987]**

A variety of chemotherapeutic agents, particularly the anthracyclines, are known to produce cardiac toxicity (1). 5-Fluorouracil (5-FU), an antimetabolite, has been in use since 1957. Its major side effects, principally gastrointestinal and hematological, have been well-described. There have been scattered reports of 5-FU cardiotoxicity in the literature (2-15), but this side effect is not as widely appreciated and is therefore frequently overlooked.

We report here three additional cases of cardiac toxicity from 5-FU and review the literature to emphasize this potential clinical problem.

## CASE REPORTS

### Patient 1

Patient 1 was a 59-year-old female with metastatic lung carcinoma who was admitted for cisplatin (Day 1) and 5-FU (1000 mg/m$^2$ iv by continuous infusion daily for 5 days) therapy. She had no history of cardiac disease. The cardiovascular system and ECG were normal on examination (fig 1A). On the fourth day of the 5-FU infusion she experienced 60 minutes of substernal chest pain radiating to the neck associated with nausea. An ECG showed atrial fibrillation (rate of 160) with ST elevations in I, III, aVF, and $V_2$-$V_6$ consistent with an acute myocardial infarction or pericarditis (fig 1B). The 5-FU was discontinued. She converted to normal sinus rhythm after administration of digoxin. Seventy-two hours later the ECG showed only minimal ST elevation and serial cardiac enzymes remained normal (fig 1C). The 5-FU infusion was restarted, but 18 hours later she again developed acute substernal chest pain, atrial fibrillation, and ST elevations (fig 1D). The ECG normalized in 48 hours, with the exception of frequent premature atrial contractions, and serial cardiac enzymes remained normal. Echocardiogram showed myxomatous disease of the mitral valve with normal left ventricular size and function.

Three weeks later the patient was admitted for a second cycle of cisplatin and 5-FU. ECG was normal with infrequent premature atrial contractions. On the second day of 5-FU infusion she again developed substernal chest pain radiating into the neck associated with nausea which resolved with sublingual nitroglycerin. The 5-FU infusion was continued. Five hours later she had recurrent severe substernal chest pain lasting 45 minutes unrelieved by nitroglycerin and morphine sulfate. The 5-FU infusion was discontinued. An ECG showed normal sinus rhythm with minimal ST elevations in I, II, and aVF. Serial cardiac enzymes were normal and the ECG returned to normal after 24 hours.

She died of progressive disease 6 months later. Autopsy revealed widely patent coronary arteries without evidence of atherosclerosis. There were no myocardial lesions suggestive of previous myocardial infarctions. There was no evidence of neoplastic involvement either of the pericardium or myocardium.

### Patient 2

Patient 2 was a 71-year-old man with carcinoma of the esophagus who had myocardial infarctions 4 and 5 years earlier and was admitted for cisplatin (Day 1

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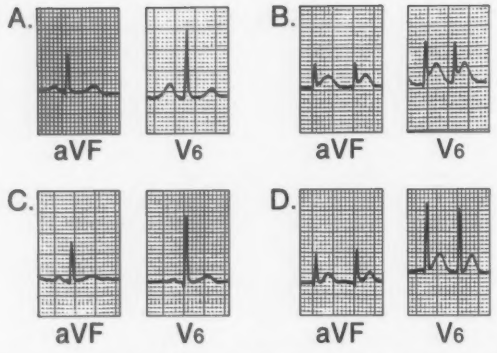


FIGURE 1.—59-year-old-female. A. Baseline ECG, leads aVF and $V_6$. B. ST elevations on 4th day of 5-FU infusion. C. ST elevations 2 hrs after discontinuation of 5-FU. D. ST elevations 18 hrs after resumption of 5-FU infusion.

only) and 5-FU (1000 mg/m$^2$ iv by continuous infusion daily for 5 days) therapy. On digoxin and transdermal nitroglycerin, he had had no angina while walking 3 miles daily. Physical examination of the cardiovascular system was normal. ECG showed normal sinus rhythm with Q waves in II, III, and aVF with T-wave inversion, poor R-wave development in $V_1$–$V_4$, and a peri-infarction block consistent with his prior infarctions. On the second day of the 5-FU infusion he developed acute substernal chest pain, rapidly became hypotensive and unresponsive, and died 48 hours later of refractory cardiogenic shock. Autopsy showed severe coronary artery disease with occlusion of the right coronary and anterior descending coronary arteries with 2-day-old acute anterior septal myocardial infarction.

### Patient 3

Patient 3 was a 67-year-old female with squamous cell carcinoma of the esophagus who was admitted for cisplatin (Day 1 only) and 5-FU (1000 mg/m$^2$ iv by continuous infusion daily for 5 days) therapy. She described a vague "coronary event" 18 years earlier and hypertension well-controlled on diuretic therapy. Physical examination of the cardiovascular system was normal. An ECG showed sinus tachycardia with left axis deviation and occasional premature ventricular contractions (fig 2A). On the second day of 5-FU therapy she developed tachycardia followed by anterior chest pain. An ECG showed loss of R waves in $V_2$–$V_6$ with deep T-wave inversions consistent with acute anterior myocardial infarction (fig 2B). The 5-FU infusion was discontinued. An echocardiogram revealed akinesis of the lower half of the septum and hypokinesis of the anterolateral wall. Serial cardiac enzymes were normal. A pyrophosphate scan revealed an extensive anterolateral and septal myocardial infarction with a left ventricular ejection fraction of 27%.

## LITERATURE REVIEW

We have reviewed 38 previous case reports describing cardiac toxicity in patients receiving 5-FU (table 1) (2–15). Patients ranged from 33 to 70 years of age (median, 57 years). There were 20 males and 16 females. Twenty-two patients received 5-FU alone, while 18 received a variety of chemotherapeutic drugs with 5-FU, primarily vinca alkaloids and nitrosoureas. All patients but one presented with anterior wall chest pain suggestive of cardiac ischemia. All but five of these episodes occurred after the second dose and most only after the third dose or day of 5-FU. Of 13 patients who were rechallenged with 5-FU, 11 again developed identical symptoms. Most patients (79%) had "ischemic" changes on their ECGs, principally ST elevations and T-wave depressions or inversions. Seven patients sustained frank myocardial infarctions, with two deaths. In addition, one patient each developed ventricular fibrillation, congestive heart failure, and tachycardia and hypotension unassociated with chest pain. Therefore, 26% of the patients (ten of 38) who had cardiac problems while receiving 5-FU died or had significant morbidity.

## DISCUSSION

In this report we report an additional three cases of significant 5-FU-related cardiac toxicity. All patients presented with anterior chest pain, suggesting cardiac ischemia, some hours after initiation of a continuous infusion of 5-FU. All of our patients received cisplatin in addition to 5-FU, although generally the cisplatin was administered days before the patient developed chest pain. The only reports of cardiac toxicity related to cisplatin have been asymptomatic electrocardiographic changes (1). Since we have not seen similar problems with patients receiving either cisplatin alone or other cisplatin-based chemotherapy combinations, we do not believe that the cardiac events described are related to cisplatin. We cannot, however, disprove the

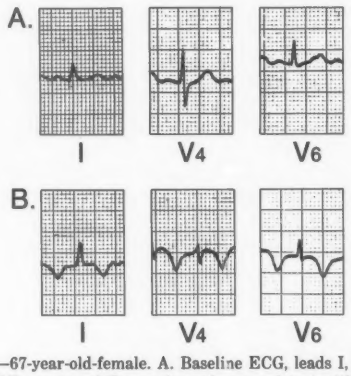


FIGURE 2.—67-year-old-female. A. Baseline ECG, leads I, $V_4$, and $V_6$. B. Loss of R waves and T-wave inversions consistent with an anterior myocardial infarction on 2nd day of 5-FU infusion.

TABLE 1.—Literature reports of substernal chest pain associated with 5-FU

| Patient No. | Sex | Age (yrs) | Previous cardiac history* | 5-FU† | | Ischemic ECG changes‡ | Other§ | Ref No. |
|---|---|---|---|---|---|---|---|---|
| | | | | Dose | Method | | | |
| 1 | F | 64 | Yes | 1st | CI | Yes | | 2 |
| 2 | F | 57 | No | 1st | CI | Yes | a | 2 |
| 3 | M | 65 | Yes | 1st | CI | Yes | a | |
| | | | | 6th | CI | Yes | b, d | 3 |
| 4 | M | 44 | No | 1st | CI | Yes | c | 3 |
| 5 | F | 51 | No | 1st | CI | No | a | 3 |
| 6 | F | 64 | No | 2nd | CI | Yes | a | 4 |
| 7 | F | 63 | No | ~12th | IVP | Yes | a, b, e | 5 |
| 8 | F | 47 | No | 2nd | IVP | Yes | | 5 |
| 9 | M | 67 | Yes | 3rd | IVP | Yes | | 5 |
| 10 | F | 66 | Yes | 4th | IVP | Yes | | 5 |
| 11 | F | 53 | Yes | 4th | IVP | Yes | | 5 |
| 12 | M | 60 | Yes | 3rd | IVP | NR | | 5 |
| 13 | F | 64 | Yes | ~6th | IVP | Yes | | 5 |
| 14 | M | 64 | No | 13th | IVP | NR | | 5 |
| 15 | M | 45 | No | 3rd | IVP | NR | | 5 |
| 16 | M | 58 | No | ~15th | IVP | NR | | 5 |
| 17 | F | 66 | No | 4th | IVP | NR | f | 5 |
| 18 | M | 49 | No | ~22nd | IVP | Yes | | 5 |
| 19 | M | 53 | No | 2nd | IVP | NR | | |
| | | | | 3rd | IVP | Yes | b, e | 5 |
| 20 | F | 69 | No | 3rd | IVP | Yes | | 5 |
| 21 | M | 60 | No | 3rd | IVP | Yes | b | 5 |
| 22 | M | 45 | No | ~23rd | IVP | Yes | | 5 |
| 23 | M | 46 | Yes | ~6th | IVP | Yes | | 5 |
| 24 | M | 33 | No | 3rd | CI | NR | | |
| | | | | 5th | CI | Yes | a, b, g | 6 |
| 25 | M | 40 | No | 10th | IVP | Yes | b, e, h | 7 |
| 26 | F | 41 | No | 2nd | CI | Yes | a | 9 |
| 27 | F | 69 | No | 3rd | IVP | NR | | 9 |
| 28 | M | 62 | No | 3rd | IVP | Yes | a, b, e | 9 |
| 29 | F | 45 | No | 2nd | IVP | Yes | a | 9 |
| 30 | M | 48 | No | 4th | IVP | Yes | | |
| | | | | 6th | IVP | Yes | | |
| | | | | 7th | IVP | Yes | i | 10 |
| 31 | M | 64 | No | 4th | CI | Yes | c, h, i | 11 |
| 32 | M | 59 | No | 9th | CI | yes | | |
| | | | | 13th | CI | Yes | b, j | 11 |
| 33 | F | 65 | No | 6th | IVP | Yes | d | 12 |
| 34 | M | 43 | No | 2nd | IVP | Yes | a | 12 |
| 35 | M | 51 | Yes | 5th | CI | NR | a, i | 13 |
| 36 | F | 43 | No | 2nd | IVP/CI | Yes | | 14 |
| 37 | F | 70 | No | 2nd | IVP | Yes | | |
| | | | | 3rd | IVP | Yes | a, d | 14 |
| 38 | F | 49 | No | 2nd | IVP | Yes | | |
| | | | | 1st | PO | Yes | | |
| | | | | 1st | CI | Yes | a | 15 |

*Positive previous cardiac history denotes history of ischemic chest pain or myocardial infarction.

†CI = continuous iv infusion; IVP = iv bolus; and PO = orally. Cardiac event observed after the dose No. indicated.

‡NR = no report of ischemic ECG changes.

§a = chest pain on repeat administration; b = abnormal cardiac enzymes; c = no chest pain on repeat administration; d = myocardial infarction, died; e = myocardial infarction, survived; f = tachycardia, hypotension; g = congestive heart failure; h = subsequent angiogram showed no coronary atherosclerosis; i = subsequent stress exercise test normal; and j = ventricular fibrillation.

possibility that the cardiac toxicity represents a synergistic effect of 5-FU and cisplatin.

Our three patients with apparent 5-FU cardiac toxicity were observed during a period of 2½ years. We also observed four additional patients during this time period in whom 5-FU may have exacerbated underlying heart disease, but the circumstances were less certain. We estimate that we treated approximately 36 patients during this time period with continuous infusions of 5-FU. Since all three patients whom we identified with cardiac toxicity were in this group of patients, this suggests an incidence of cardiac toxicity of approximately 10% in patients receiving continuous infusions of 5-FU. A subsequent study by the Lung Cancer Study Group incorporating continuous-infusion 5-FU also reported two cardiac events (myocardial infarction and fatal hypotension) in 20 patients (10%) with Stage III non-small cell lung cancer (16). On the other hand, we did not recognize any episodes of cardiac toxicity among the approximately 120 patients who received iv bolus doses of 5-FU during this time period, suggesting an incidence of cardiac toxicity of $< 1\%$ with bolus administrations of 5-FU. Similar conclusions were reached by Labianca et al (5), who reviewed 1083 patients who had received 5-FU and found 17 cases of 5-FU cardiac toxicity, an incidence of 1.6%. In the subset of patients who had a history of previous cardiac disease, 4.5% (six of 131 patients) subsequently developed chest pain after administration of 5-FU compared to only 1.1% (11 of 952) of patients without a history of heart disease.

The mechanism of cardiotoxicity of 5-FU is not established. Radiolabeled 5-FU accumulates in the myocardium of rodents (17) and a myocarditis/pericarditis after administration of iv 5-FU has been observed (18–20), suggesting that 5-FU may directly damage cardiac cells. Alternatively, a complex of 5-FU and cardiac cells or other tissue components may stimulate a myotoxic autoimmune response. However, the clinical features observed suggest cardiac ischemia rather than direct myocardial toxicity. Experimental support for this mechanism comes from the work of Matsubara et al (21), who administered 5-FU iv to guinea pigs and produced ischemic ECG changes. The recurrent nature of the chest pain, the reproducible ECG abnormalities, and the pain in temporal relationship to 5-FU suggest a dynamic mechanism, such as coronary artery spasm (5,6,9,11,13–15,22). The clinical data indicate that classical atherosclerosis is not necessarily involved, ie, our first patient, Patients 25 and 31 in table 1, who had normal cardiac angiograms, and Patients 31 and 35, who had normal exercise stress tests. If the underlying mechanism of 5-FU-related cardiac toxicity is coronary artery spasm, then reasonable prophylaxis or treatment would be the use of nitrates and calcium channel blockers.

Regardless of the specific mechanism, occasional patients will develop cardiac ischemia while receiving 5-FU, an occurrence perhaps tenfold more likely if the 5-FU is administered by continuous infusion. A proportion of these patients may experience significant morbidity or even mortality as a result. The likelihood of recurrence of symptoms is high on subsequent administration of the drug. It seems likely that pre-existing cardiac disease increases the risk of serious sequelae of 5-FU-related cardiac toxicity. Therefore, in patients with significant coronary artery disease, we suggest the use of alternative methods of treatment.

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# Azacitidine: 10 Years Later[1]

**Alice B. Glover, Brian R. Leyland-Jones,* Hoo G. Chun, Barbara Davies, and Daniel F. Hoth[2]**

**Azacitidine has been undergoing clinical trials for almost 20 years and is internationally considered to have a useful place in the treatment of acute nonlymphocytic leukemia. However, its role in the various combinations for induction, intensification, maintenance, or relapse regimens has not yet been clearly defined. This review outlines the last 10 years' clinical experience with the drug in acute nonlymphocytic leukemia, analyzes what critical information has yet to be obtained, and suggests what phase III trials may still be feasible to gather that information. [Cancer Treat Rep 71:737-746, 1987]**

Azacitidine is a ring analog of the natural pyrimidine nucleoside cytidine synthesized in 1964. Clinical trials began in Europe in 1967 and in the United States in 1970. Preclinical information and results of early clinical trials were comprehensively discussed in a previous review (1). In that article the authors described a drug with "consistent antitumor activity in patients with acute myelogenous leukemia resistant to previous treatment" and "an overall response rate of 36% with 20% complete remissions . . . in . . . acute myelogenous leukemia . . . of adults and children." Furthermore, the median duration of remission with azacitidine was noted to be "encouragingly long." In summary, Von Hoff et al (1) suggested four potential directions for the development of azacitidine as an antileukemic agent, as follows: (*a*) to define the use of azacitidine in the first-line treatment of acute nonlymphocytic leukemia (ANLL); (*b*) to explore in a controlled manner the use of infusions of the drug versus other modes of administration; (*c*) to examine the use of sc administration, since this would be a more desirable route, especially in children; and (*d*) to define the role of azacitidine in consolidation, maintenance, and late intensification regimens.

The review also suggested two other areas of exploration: (*a*) to develop the early observations of the protective effect of azacitidine on mice exposed to lethal x-irradiation; and (*b*) to expand animal experiments using azacitidine with cytidine as an antidote, to improve the therapeutic index of the drug.

After a brief update on preclinical issues, this paper will examine the last 10 years' clinical development of azacitidine, summarize how these six recommendations have been pursued, and finally examine what still needs to be done to establish the role of azacitidine in the treatment of ANLL.

## MECHANISM OF ACTION

Several steps in cellular metabolism are affected by azacitidine and previous papers (1–5) have dealt with these in some detail. The accompanying paper on the biochemistry of azacitidine reviews these data at length (6) and only the main features are summarized here.

Azacitidine is transported into the cell and sequentially phosphorylated by the same mechanisms as for cytidine and uridine. 5-Azacytidine triphosphate is incorporated into RNA, disrupting the synthesis and processing of both nuclear and cytoplasmic species and leading to inhibition of protein synthesis. It is also incorporated to a lesser extent into DNA leading to inhibition of DNA synthesis.

The most interesting development at the biochemical level since previous reviews (1–3) has been the increasing use of azacitidine as a "laboratory tool" to examine the role played by DNA methylation upon gene expression and activation. After incorporation into DNA, azacitidine noncompetitively inhibits DNA methyltransferase, causing a block in cytosine methylation in newly replicated DNA (7–12). There is now a large body of evidence, which has been comprehensively reviewed (13–18), indicating that alterations in the pattern of cytosine methylation are causally related to gene expression and cell differentiation. This hypomethylation of DNA is thus probably the mechanism underlying the differentiating activity of azacitidine (19) and may be related to its tumorigenic effects (20–27).

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## PHARMACOKINETICS

The initial study by Troetel et al (28) had shown that the plasma half-life of total radioactivity (parent drug plus metabolites) after iv administration was about 3½ hours. Subsequently, Israili et al (29) obtained similar results for total radioactivity. In addition, they identified at least two metabolites and/or decomposition products of azacitidine in plasma. They also showed that levels of the parent drug declined much more rapidly than those of total radioactivity so that in one patient levels of azacitidine were < 2% of total radioactivity after 30 minutes. Renal clearance of radioactivity varied from 74 to 210 ml/minute in the five patients in Israili's study and most of the administered radioactivity was excreted via the urine.

From preclinical studies it is known that azacitidine is rapidly deaminated by cytidine deaminase which is present in serum as well as in the liver, granulocytes, and gastrointestinal epithelium. This enzyme can be inhibited both in human leukemic cells in vitro and in the mouse in vivo by tetrahydrouridine (30,31). The relative role and clinical significance of deamination in the metabolism of azacitidine in humans as compared with other enzymatic degradation pathways cannot be determined until a specific and sensitive assay for azacitidine is developed.

## RESULTS OF CLINICAL TRIALS

This paper will only review those trials of azacitidine conducted in ANLL of adults and children. Phase II trials in several solid tumors including colorectal, breast, lung, melanoma, head and neck, and renal carcinoma did not reveal adequate activity to justify further investigation in these tumor types. A summary of these trials is shown in table 1.

TABLE 1.—AZA in solid tumors (phase II trials only)*

| Disease site (group) | Dose ($mg/m^2$) | Schedule | Tumor | No. of — Evaluable patients (No. entered) | No. of — Responses | Ref No. |
|---|---|---|---|---|---|---|
| Advanced gastrointestinal cancer (Mayo) | 750 | Daily × 5 or daily × 10 | Colon cancer | 27 | 1 PR | 32 |
| | | | Pancreatic cancer | 1 | 1 < PR | |
| | | | Gastric cancer | 1 | | |
| Breast cancer (RPMI) | 60<br>100 | Daily × 10<br>then 2 ×/wk | | 27 | 2 PR | 33 |
| Broad phase II (COG) | 60 | Daily × 10† | Breast cancer | 29 | 6 PR | 34 |
| | | | Lung cancer | 24 | 1 PR | |
| | | | Large cell cancer | | | |
| | | | Intestinal cancer | 26 | 0 | |
| | | | Melanoma | 12 | 0 | |
| | | | Hodgkin's disease | 6 | 1 PR | |
| | | | NHL | 8 | 2 PR | |
| | | | Other cancer | 59 | 9 PR | |
| Broad phase II (SWOG) | 225 | Daily × 5 every 3 wks | Colon cancer | 15 | 0 | 35 |
| | | | Renal cancer | 17 | 0 | |
| | | | Breast cancer | 14 | 0 | |
| | | | NSCLC | 12 | 2 PR | |
| | | | SCLC | 14 | 1 PR | |
| | | | Testicular cancer | 4 | 2 PR | |
| | | | Melanoma | 13 | | |
| | | | Other cancer | 85 | | |
| Broad phase II (SEG) | 150 | 2 ×/wk for 6 wks | Breast cancer | 6 (8) | 0 | 36 |
| | | | Renal cancer | 10 (12) | 1 PR | |
| | | | Colon cancer | 7 (9) | 0 | |
| | | | Melanoma | 10 (10) | 1 < PR | |
| | | | SCLC | 2 (3) | 0 | |
| | | | NSCLC | 26 (33) | 1 PR; 1 < PR | |
| | | | H and N | 14 (14) | 0 | |
| | | | Other cancer | 16 (24) | 0 | |
| Broad phase II (Mt. Sinai) | 30–120<br>25–200 | 24-hr CIV<br>+<br>Pyrazofurin | Other cancer | 6 | 0 | 37 |
| Sarcoma | 150–200 | Daily × 5 | Osteogenic sarcoma | 7 | 0 | 38 |
| | | | Ewing's sarcoma | 7 | 0 | |

*AZA = azacitidine; Mayo = Mayo Clinic; RPMI = Roswell Park Memorial Institute; COG = Central Oncology Group; SEG = Southeastern Cancer Study Group; Mt. Sinai = Mt. Sinai Hospital; CIV = continuous iv infusion; NHL = non-Hodgkin's lymphoma; NSCLC = non-small cell lung cancer; SCLC = small cell lung cancer; H and N = head and neck cancer; PR = partial response; and < PR = objective response not meeting criteria for PR.
†= 1.6 mg/kg.

TABLE 2.—AZA alone in ANLL*

| Group or institution | Dose ($mg/m^2$) | Schedule | No. of— Evaluable patients T | Rl | Rf | CRs T | Rl | Rf | PRs T | Rl | Rf | Ref No. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SWOG† | 100<br>750<br>75 | Every 8 hrs × 15<br>Single iv<br>4-hr infusion every 6 hrs × 20 | 50 | 50 | | 10 | 10 | | 5 | 5 | | 39 |
| SWOG† | 250<br>300<br>200<br>200<br>150 | Iv every 4 hrs × 3<br>Daily CIV × 5<br>Daily CIV × 5<br>Daily CIV × 7<br>Daily CIV × 10 | 101 | 101 | | 8 | 8 | | | | | 40 |
| MDA | 150-600 | Daily iv × 5 | 18 | 18 | | 3 | | | 4 | | | 41 |
| MSKCC | 120-300 | Daily iv × 5 | 6 | | | 0 | | | 1 | | | 42 |
| BCRC | 200-250 | Daily iv × 5 | 18 | 14 | 4 | 5 | 3 | 2 | 1 | | | 43 |
| WCG | 60<br>100<br>180<br>300 | Every 8 hrs × 15<br>Every 8 hrs × 15<br>Daily iv × 5<br>Daily iv × 5 | 6<br>23<br>5<br>21 | | | 0<br>3<br>1<br>2 | | | 2<br>4<br>0<br>7 | | | 44 |
| SEG | 150-200 | Daily CIV × 5 | 45 | | | 11 | | | 4 | | | 45 |
| University of Chicago | 200 | Daily iv × 5 | 15 | 9 | 6 | 5 | 3 | 2 | 2 | 2 | 0 | 46 |
| MMC | 150 | Daily CIV × 5 | 10 | | 10 | 3 | | 3 | 3 | | 3 | 47 |
| ECOG | 50-90 | 3-hr infusion every 8 hrs × 15 | 3 | — | 3 | 0 | — | — | 0 | — | — | 48 |
| Subtotal | | | 321 | 174 | 23 | 51 | 24 | 7 | | | | |
| CCSG | 150-200 | Daily iv × 5 | 14 | | 14 | 5 | | 5 | 1 | | 1 | 49 |
| Total | | | 335 | | 56 | | | | | | | |

*MDA = M. D. Anderson Hospital and Tumor Institute; MSKCC = Memorial Sloan-Kettering Cancer Center; BCRC = Baltimore Cancer Research Center; WCG = Western Cancer Study Group; MMC = Maine Medical Center; ECOG = Eastern Cooperative Oncology Group; CCSG = Children's Cancer Study Group; T = total No. of evaluable patients who were considered by the investigators to have had an adequate trial of azacitidine; Rl = No. of patients relapsed after induction on standard therapy; Rf = No. of patients refractory to standard induction regimens; and CR = complete response.

†Breakdown by infusion schedule included all leukemia types, so only total for ANLL given here.

### Azacitidine as a Single Agent in ANLL

Table 2 shows the azacitidine single-agent data from trials sponsored by the National Cancer Institute as of June 1985. Some of these studies included acute lymphocytic leukemia and blast crisis of chronic myelocytic leukemia, but the data presented in table 2 are only for ANLL. The single-agent data base shown in the earlier review (1) has now been expanded from a total of 200 patients to a total of 335. The overall complete response rate has fallen from 20% to 16.7%. As can be seen, the pediatric data base has not been expanded in single-agent use since 1976. Furthermore, the largest study (40) observed only eight complete responses among 101 evaluable patients who received an adequate trial of azacitidine.

### Effect of Schedule on Activity of Azacitidine

Azacitidine was active on both daily and intermittent schedules against L1210 leukemia. While its predominant effects were in the S phase, its action was not limited to the S phase (3). In early clinical trials three schedules were used: daily × 5; every 8 hours × 15; and daily continuous infusion × 5–7 days. Because of the instability of the drug most of the continuous-infusion studies actually employed a 3-hour infusion every 8 hours (40,45,47). [There has been conflicting advice in various publications about the stability of azacitidine in iv solutions. This issue has recently been reviewed (50) and a comparative analysis of decomposition rate in several infusion solutions carried out (51). The recommendation from these studies is that the reconstituted solution be further diluted within 30 minutes in lactated Ringer's injection, USP, which provides optimum pH for stability. Under these conditions there will be 90% of intact drug remaining after 2 hours and 80% after 6 hours. Therefore, the dilute solution should be infused over ≤ 2–3 hours.]

Table 3 summarizes the response rate in acute leukemia according to the three schedules used in the initial trials. Since data are pooled from all the trials in table 2, the analysis includes not only patients with ANLL

TABLE 3.—Effect of schedule on AZA response rate*

| Dose ($mg/m^2$) | Schedule | No. of evaluable patients | Response rate (%) CR | PR |
|---|---|---|---|---|
| 150–500 | Daily × 5 | 122 | 21 | 17 |
| 50–100 | Every 8 hrs × 15 | 110 | 24 | 8 |
| 150–300 | Daily CIV × 5–7 | 138 | 23 | 9 |

*Data are pooled from all the trials in table 2; therefore Nos. include not only patients with ANLL but also patients with acute lymphocytic leukemia and blast crisis of chronic myelocytic leukemia.

but also patients with acute lymphocytic leukemia and blast crisis of chronic myelocytic leukemia. Using this form of analysis to achieve large denominators, the complete response rate does not vary between any of these schedules. However, the question of schedule dependence has not been addressed in an appropriate prospectively randomized trial with statistically defined endpoints.

It is difficult to assess from pooled data whether toxicity is schedule-dependent. The Southwest Oncology Group (SWOG) study (40) tested several different schedules sequentially. Dose and schedule were altered de-

TABLE 4.—AZA: 2-drug combination regimens in remission induction of ANLL*

| Group or institution | Regimen | Dose ($mg/m^2$) | Schedule | No. of— Evaluable patients T | Rl | Rf | CRs T | Rl | Rf | PRs T | Rl | Rf | Ref No. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | *Adult* | | | | | | | | | | |
| BCRC | AZA<br>MGBG | 33<br>100 | Every 8 hrs × 15<br>Daily CIV × 5 | 8 | 4 | 4 | 0 | | | 0 | | | 52 |
| BCRC | AZA<br>PF | 150–250<br>7.5–30 | Daily × 5<br>Daily × 1 | 16 | | | 2 | | 2 | 1 | 1 | | 53 |
| SEG | AZA<br>PF | 150<br>25–150 | Daily CIV × 5<br>Single iv | 27 | | | 3 | | | 4 | | | 54 |
| SEG | AZA<br>TGdR | 150<br>300 | Daily CIV × 5<br>Daily × 5 | 81 | 81 | | 16 | 16 | | 5 | 5 | | 55 |
| CALGB | AZA<br>Zorubicin | 50<br>75 | 3-hr infusion every day × 5<br>Daily × 3 | 29 | 10 | 19 | 8 | 7 | 1 | | | | 56 |
| SEG | AZA<br>AMSA | 112–200<br>75–150 | Daily CIV × 4<br>Daily × 4 | 27 | | 27 | 3 | | 3 | | | | 44 |
| | AZA<br>AMSA | 200<br>150 | Daily CIV × 4<br>Daily × 4 | 53 | | 53 | 7 | | 7 | 5 | | 5 | 57 |
| ECOG | AZA<br>AMSA | 150<br>150 | Daily × 5<br>Daily × 5 | 18 | 12 | 6 | 7 | 7 | 0 | | | | 58 |
| BCRC | AZA<br>VP-16 | 50<br>75 | Every 8 hrs × 15<br>Daily × 5 | 14 | 10 | 4 | 0 | | | 0 | | | 59 |
| NWU | AZA<br>AMSA | 150<br>150 | Daily × 5<br>Daily × 5 | 16 | 4 | 12 | 1 | 0 | 1 | | | | 44 |
| DFCC | AZA<br>VP-16 | 300<br>200 | Daily × 2<br>Daily × 3 | 13 | | 13 | 0 | | | 0 | | | 44 |
| | | | *Pediatric* | | | | | | | | | | |
| DFCC | AZA<br>VP-16 | 300<br>200 | Daily × 2<br>Daily × 3 | 14 | 0 | 14 | 3 | 0 | 3 | 2 | 0 | 2 | 44 |
| St. Jude† | AZA<br>VP-16 | 150<br>100 | Daily × 2<br>Daily × 3 | 16 | | 16 | 1 | | 1 | | | | 60 |
| | AZA<br>VP-16 | 300<br>200 | Daily × 2<br>Daily × 3 | 14 | | 14 | 6 | | 6 | | | | 61 |
| St. Jude† | AZA<br>VP-16 | 300<br>250 | Daily × 2<br>Daily × 3 | 6 | | 6 | 6 | | 6 | | | | 62 |
| POG† | AZA<br>VP-16 | 300<br>250 | Daily × 2<br>Daily × 3 | 52 | 36 | 16 | 28 | 17 | 11 | 3 | 3 | 0 | 63 |
| CCSG | AZA<br>DNR | 100<br>30 | Daily × 5<br>Daily × 3 | 34 | | 34 | 14 | | 14 | 4 | | 4 | 44 |

*CALGB = Cancer and Leukemia Group B; NWU = Northwestern University; DFCC = Dana-Farber Cancer Center; St. Jude = St. Jude Children's Hospital; POG = Pediatric Oncology Group; MGBG = mitoguazone; PF = pyrazofurin; TGdR = deoxythioguanosine; AMSA = amsacrine; VP-16 = etoposide; and DNR = daunorubicin.

†Ongoing.

pending on the toxicity in the preceding group of patients. They found that the proportion of severe nausea and vomiting was significantly less in patients receiving the low-dose infusion schedules (150–200 mg/m$^2$/day in four divided doses) rather than the high once-weekly dose (750 mg/m$^2$ in three divided doses). In this trial there were several cases of coma. Upon review of each case it was concluded that some may be partially attributable to azacitidine. The incidence rate in the higher-dose schedules (300 and 750 mg/m$^2$/day in divided doses) was 22%, whereas that on the 150–200-mg/m$^2$/day regimens was 4.6%. Thus, the lower-dose infusion schedules appear to be associated with less gastrointestinal and neurological toxicity. These lower-dose schedules have been almost universally adopted for the more recent studies.

### Azacitidine in Two-Drug Combinations in Relapsed or Refractory ANLL

Table 4 shows an analysis of the two-drug combination regimens separated into refractory and relapsed patients and into adult and pediatric populations. The total number of evaluable patients with ANLL who have received azacitidine in combination with one other drug as second-line or later therapy is now > 360. Two observations are immediately apparent. First, the patient denominator on each regimen (with the possible exception of azacitidine + deoxythioguanosine) is inadequate to demonstrate superiority of any one combination over another. Second, the denominators are again inadequate to show superiority of any combination over the single-agent results. Even after extracting the data for the most frequently used combination in adults (azacitidine and amsacrine) from the three phase II trials, there are still only 15 complete responses and five partial responses among 87 patients. In pediatrics, the combination of azacitidine with etoposide was the most frequently tested. An overall complete response rate of 50% using these two drugs was seen in the phase II evaluation and complete responses were observed in both relapsed and refractory patients. None of these two-drug combinations has been brought into front-line therapy.

### Azacitidine: Multidrug Combinations

Table 5 presents these data subdivided by adult and pediatric trials. Of particular note in the pediatric trials is the D-ZAPO (daunorubicin, azacitidine, cytarabine, prednisone, and vincristine) combination (65,66) which is the only azacitidine combination brought into front-line therapy in any leukemia trial, either adult or pediatric. The early promise of this combination suggested in the 1976 review (12 complete remissions among 24 patients) has been substantiated by the much greater patient denominator now on this trial (117 complete remissions among 163 patients). However, the superiority of D-ZAPO over regimens which do not include azacitidine remains to be substantiated in a randomized trial.

The MAZE (amsacrine, azacitidine, and etoposide) combination (67,68) has been tested in very few patients to date; the numbers are inadequate to substantiate whether the difference between the Medical Research Council (MRC) trial (seven complete responses among

TABLE 5.—AZA: multidrug combinations in remission induction of ANLL*

| Group or institution | Prior treatment | Regimen | Dose (mg/m$^2$) | Schedule | No. of— Evaluable patients | CRs | PRs | Ref No. |
|---|---|---|---|---|---|---|---|---|
| | | | | *Pediatric* | | | | |
| SWOG | Rel and Ref | AZA | 150 | 8-hr infusion daily × 4 | 56 | 8 | 7 | 64 |
| | | VCR | 1.5 | Single dose | | | | |
| | | Pred | 60 | Daily × 4 | | | | |
| CCSG† | No | DNR | 30 | Daily × 3 | 163 | 117 | | 65, 66 |
| | | AZA | 50 | Every 12 hrs × 8 | | | | |
| | | Ara-C | 25 | Every 8 hrs × 12 | | | | |
| | | Pred | 40 | Daily × 4 | | | | |
| | | VCR | 1.5 | Daily × 1 | | | | |
| | | | | *Adult* | | | | |
| BCRC | Rel and Ref | AZA | 50 | Every 8 hrs × 15 | 15 | 2 | 1 | 59 |
| | | VP-16 | 50 | Daily × 5 | | | | |
| | | VBL | 6 | Days 1 and 6 | | | | |
| MRC | 1 | AZA | 100 | Daily × 6 | 10 | 7 | | 67 |
| | | AMSA | 100 | Daily × 6 | | | | |
| | | VP-16 | 100 | Daily × 6 | | | | |
| SWOG | 2 (1-5) | AZA | 100 | Daily CIV × 5 | 17 | 3 (15 marrow hypoplasia) | | 68 |
| | | AMSA | 60 | Daily × 5 | | | | |
| | | VP-16 | 30 | Daily × 5 | | | | |

*VCR = vincristine; Pred = prednisone; ara-C = cytarabine; VBL = vinblastine; Rel = relapsed; and Ref = refractory.
†Ongoing.

ten patients) and the SWOG trial (three complete responses among 27 patients) is genuine. The difference in response rate may be due to selection of a lower dose of amsacrine and etoposide in the SWOG trial. Patient selection could also be a factor, since all patients in the MRC trial were in first relapse, whereas the median number of previous treatment regimens in the SWOG trial was two.

### Azacitidine: Consolidation and Maintenance Regimens

Table 6 lists the trials in which azacitidine has been incorporated into front-line consolidation and maintenance regimens. Most of these trials are ongoing. None of these trials was designed to address the role of azacitidine as an individual agent. The VAPA protocol (71,72) used early and late intensification and sequencing of different chemotherapy combinations (which included azacitidine) after remission induction with a standard combination of cytarabine, daunorubicin, vincristine, and prednisone. The aim of the intensive sequential regimen was to achieve adequate reduction of residual leukemic cells and prevent the emergence of drug-resistant cells. When last reported, with a median follow-up of 43 months (72), there was a complete remission rate of 70% and a 3-year actuarial probability remission duration of 56% for patients < 18 years of age and of 45% for patients 18–50 years of age. The D-ZAPO protocol (65,66) brought azacitidine into front-line remission induction regimens in pediatric ANLL. It also continued its use in both arms of a randomized maintenance program, comparing a more intensive schedule with a less intensive schedule (daily rather than intermittent 6-thioguanine and cyclophosphamide added to the monthly pulses of cytarabine and azacitidine and earlier administration of intrathecal methotrexate). The more intensive regimen showed a significantly improved duration of marrow remission, although survival was similar for both schedules. The results of the SEG study using azacitidine in two of the three arms of a randomized consolidation study in adult ANLL are so far only available in abstract form (69,70). In this study there was no advantage to adding beta-deoxythioguanosine to the azacitidine arm in terms of percentages of patients remaining in complete remission.

## DISCUSSION

The development of azacitidine over the last 10 years has been disappointing in its lack of systematic progression. Of the four goals delineated in the earlier review for azacitidine in ANLL, only one has been partially achieved, namely, the demonstration that there is no major difference in response rate between the three schedules of administration. However, even this conclusion is based on pooled data from trials in all forms of leukemia. The other three goals have not been achieved: (*a*) except for one series of pediatric trials, azacitidine has not been brought into first-line treatment; (*b*) the sc route of administration has not been explored; and (*c*) the role of azacitidine in consolidation, maintenance, and intensification regimens has not been defined.

Over 400 patients have been entered in two-drug combination trials, but no attempt has been made to demonstrate in a rigorous manner the superiority of any one combination over another.

Promising preclinical leads such as the use of cytidine as a rescue agent (73) and enhancement of in vitro cytostatic activity by thymidine and deoxycytidine (74) have not been pursued systematically. The radioprotective effect of azacitidine noted in mice (75) has not been investigated further except for one study of combined teratogenic effects on the mouse central nervous system (76) and one study of chromosomal aberrations in the broad bean (77). Azacitidine was not demonstrated to be protective in either of these studies. However, the original study of Vesely et al (75) undoubtedly needs replication and extension since it has significant clinical implications if confirmed.

In summary, the development of azacitidine, since its promising biological activity was first described > 20 years ago (78), has not established a definitive role in either front-line or salvage therapy of ANLL. The consequence is that azacitidine has not become generally available to physicians as a marketed drug for the treatment of leukemia.

These problems in the development of azacitidine exemplify the difficulties inherent in the development of an antileukemic agent which has shown "promising" activity in phase II trials. As seen from tables 1 and 3, considerable expenditure of patients and investigators' resources can easily occur without conclusive results being obtained.

We would like to propose a sequence of priorities for antileukemic drug development so as to achieve optimal use of all clinical and economic resources. (*a*) Pharmacokinetic parameters (particularly clearance and elimination half-life) need to be established as early as technically possible in the drug's development so that optimal schedules for phase II and III trials can be developed. In the case of azacitidine the development of a specific and sensitive assay for azacitidine had special problems due both to its chemical instability in aqueous solution and susceptibility to enzyme degradation in serum (4). Progress towards a useful assay in biological fluids was made 10 years ago (79) but this has not been developed further.

(*b*) The aim of single-agent trials of an investigational antileukemic drug in relapsed or refractory patients is to differentiate an active agent from an inactive one and to determine within reasonable confidence limits whether the response rate warrants further investigation of the drug in this disease. Since these trials provide little information about the true therapeutic efficacy of the drug, it is important that the number of patients be kept to the minimum necessary to provide a reliable basis for decision making. The standard approach has been to enter enough patients to establish a response rate for the agent in relapsed or refractory patients. The

TABLE 6.—AZA: use in front-line intensification regimens after remission induction*

| Group or institution | Protocol No. | Ref No. | Induction regimens | Intensive consolidation/maintenance/postremission-induction regimen† |
|---|---|---|---|---|
| | | | *Adult* | |
| SEG | | 69, 70 | Ara-C and DNR | AZA (× 3)<br>or<br>AZA and TGdR (× 3)<br>or<br>6TG, ara-C, and DNR |
| ECOG | EST P-B477 | 44 | Ara-C and DNR | AZA, then DNR, then ara-C (× 2) |
| SEG | AML 81-312‡ | | Ara-C and DNR | Ara-C and DNR<br>or<br>Ara-C and DNR, then AMSA, then AZA, 6TG, and DNR<br>or<br>AZA, 6TG, and DNR, then allogeneic BMT |
| DFCC | D81-018-590 | | Ara-C and DNR | DNR, ara-C, 6TG, and i.t. ara-C (× 4), then DNR, AZA, and i.t. ara-C (× 4), then ara-C, 6TG, and i.t. ara-C (× 4) |
| Baylor | D81-044-077‡ | | Ara-C, DNR, and 6TG | DNR + ara-C, then VP-16 + AZA |
| Hahnemann Medical College | D82-061-724‡ | | Ara-C, DNR, and 6TG | AMSA, AZA, and ara-C<br>or<br>Ara-C + AMSA, then AMSA + AZA |
| Stanford | D82-051-605‡ | | Ara-C, DNR, and 6TG | VP-16, AMSA, and AZA |
| | | | *Adult and pediatric* | |
| Weinstein et al | | 71, 72 | VAPA twice | DNR and ara-C (× 4), then DNR and AZA (× 4), then VCR, MePred, MP, and MTX (× 4), then ara-C (× 4) |
| | | | *Pediatric* | |
| CCSG | | 73 | D-ZAPO | 6TG, AZA, ara-C, and VCR<br>or<br>6TG, AZA, ara-C, VCR, and immunotherapy |
| CCSG | | 74 | D-ZAPO | 6TG, ara-C, and VCR<br>or<br>6TG, AZA, ara-C, VCR, and CPM |
| CCSG | CCG-251 | | Ara-C and CNR | 6TG, ara-C, and i.t. MTX (*or* allogeneic BMT), then 6TG, VCR, ara-C, AZA, and CPM (repeated continuous)<br>or<br>DNR and ara-C, then Pred and VCR, then AZA and DNR, then BCNU and CPM, then 6TG and ara-C |
| CCSG‡ | | | Ara-C and DNR<br>or<br>Ara-C, DNR, VP-16, 6TG, and DXR | Ara-C and Asp, then 6TG, VCR, ara-C, and CPM, then VP-16, DNR, ara-C, AZA, and CPM, then VP-16, DNR, ara-C, DXR, and 6TG |
| POG | 8101‡ | | Ara-C and DNR<br>or<br>Ara-C, VCR, and Dex§ | 6TG, ara-C, and AZA, then ara-C and 6TG, then VCR, ara-C, CPM, and Pred<br>or<br>DNR and ara-C, then ara-C and 6TG, then 6TG, ara-C, and AZA, then VCR, ara-C, CPM, and Pred |
| DFCC | T84-0245‡ | | HD ara-C, DNR, and i.t. ara-C, then ara-C, DNR, and i.t. ara-C | VP-16, AZA, and i.t. ara-C (× 3), then ara-C and DNR |
| Hawaii Cancer Center | D81-011-693‡ | | Ara-C, DNR, VCR, and Pred | DNR and ara-C, then DNR and AZA, then VCR, MePred, MP, and MTX, then ara-C |
| MRC | T83-1410 | | DNR, ara-C, and 6TG | DNR, ara-C, 6TG, then CPM, VCR, and ara-C,<br>or<br>AMSA, AZA, and VP-16, then ara-C and 6TG every 4 wks, then CPM, VCR, and ara-C every 4 wks |

*6TG = 6-thioguanosine; BMT = bone marrow transplant; i.t. = intrathecal; MePred = methylprednisone; MO = mercaptopurine; MTX = methotrexate; CPM = cyclophosphamide; BCNU = carmustine; DXR = doxorubicin; Asp = asparaginase; Dex = dexamethasone; and HD = high dose.

†Consolidation has not been separated from maintenance since in many protocols different working definitions are used, and in recent practice these amount to intensive postremission-induction regimens of comparable durations.

‡Ongoing study or final report not yet published.

§Arm was closed once lesser efficacy was established.

small numbers in such trials (as exemplified for azacitidine in table 2) mean that the confidence limits for the estimated response rate are very broad. For example, if three responses are observed among 25 patients, the bounds of the 95% confidence interval are 3% and 30%. In other words, a conclusion as to whether the drug is active or inactive usually cannot be reached from such a result in a phase II trial. A more appropriate design to achieve the aim of the trial would be a Fleming two-stage design (80). This design obliges the investigator before starting the trial to specify what response rate would be of sufficient interest to take the drug into combination phase II and possibly phase III studies as part of a front-line combination regimen. The design also allows for early termination should the observed response rate be below a predetermined minimum level of interest.

(*c*) In leukemia the new agent will need to be tested as part of a combination as soon as its single-agent activity has indicated that further development of the drug is warranted. At this stage a randomized pilot phase III trial using a sequential design may be appropriate (81–84). The aims of such a design are to determine the level of activity of a combination; to minimize the number of patients entered if early results indicate that the test combination is unlikely to produce a clinically meaningful improvement over a standard combination; and to avoid overinterpretation of unrandomized phase II studies in highly selected patients.

(*d*) Establishing the specific contribution of the drug to front-line combination regimen is extremely difficult in a disease like leukemia, with multiple effective agents of comparable activity being used in several different combination regimens for induction and salvage. It is important to establish whether the new agent is merely equivalent to existing agents or constitutes a significant medical advance. The clearest definition of a drug's contribution is obtained from a randomized trial in which the new agent is the only variable between the two arms. There are two possible experimental arms. In one arm, the new drug substitutes for a standard drug of similar mechanism of action and level of activity. In the second arm, the new agent is added to the standard induction regimen. The control arm in each case would be a standard induction regimen. This approach assumes that there will be small differences in effectiveness between arms; any design should include early stopping rules in the event that one arm is substantially superior.

Azacitidine has been brought into the D-ZAPO front-line combination with a promising remission rate (72%) in pediatric ANLL (65,66). However, without a randomized comparison of this regimen versus the same group of drugs minus azacitidine (ie, daunorubicin, cytarabine, prednisone, and vincristine) and no additional differences between the two arms in terms of intensification or maintenance regimens, it is impossible to say whether azacitidine materially adds to the complete remission rate or to survival.

The risk of not proceeding in a sequential fashion is that new drugs of poorly defined activity will add toxicity without additional efficacy or may compromise the efficacy of standard regimens because of reductions in dose intensity. These and other issues involved in the development of combination chemotherapy are explored in some detail by Wittes and Goldin (85).

(*e*) Similarly, the place of individual drugs in consolidation and maintenance regimens can only be reliably established by a stepwise progression comparing a standard current intensification regimen to one incorporating the new agent without compromising dose intensity of the standard regimen.

With respect to azacitidine, it is no longer feasible to perform this sequence of trials. However, the following trials are proposed as ways in which a conclusion could be reached as to whether the drug is a safe and effective component of standard leukemia therapy. (a) Use of azacitidine in front-line therapy in addition to a standard induction combination should be compared with the standard induction combination alone. The success of the D-ZAPO regimen (65,66) indicates that this could be done without compromising the total dose of the standard induction agents to a major extent. This dose of cytarabine in these trials was 25 mg/m$^2$ every 8 hours for 12 days, but that of the other agents was the same as in induction therapy without azacitidine. (b) A trial should be conducted using a combination such as MAZE which has appeared promising in noncomparative trials and evaluating the role of azacitidine by doing a randomized trial of the two-drug versus three-drug combination in first relapse. It is encouraging that this concept is being piloted for toxicity by the Pediatric Oncology Group.

The essential feature of these trials is the randomized addition design which would enable a conclusion to be reached about the role of azacitidine without compromising progress in the treatment of leukemia.

Another approach would be a randomized phase III trial to compare an alternating non-cross-resistant regimen of daunorubicin, cytarabine, and thioguanine (DAT), then MAZE with repeated cycles of DAT alone. This approach would not clarify the role of azacitidine itself. However, it would establish the role of a promising combination in front-line therapy. It would also address the more general question of the value of intensive non-cross-resistant regimens alternating in front-line therapy.

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BRIEF REPORTS

# Phase II Study of Recombinant Alfa-2a Interferon in Patients With Advanced Bone Sarcomas[1,2]

John H. Edmonson,[3,*] Harry J. Long,[3] Stephen Frytak,[3] William A. Smithson,[3] and Loretta M. Itri[4]

**Twenty previously treated patients with advanced bone sarcomas received thrice weekly im $50 \times 10^6$ IU/m$^2$ doses of human alfa-interferon (interferon alfa-2a, recombinant; Roche). Seventeen patients had metastatic osteosarcomas and one each had fibrosarcoma, mesenchymal chondrosarcoma, and malignant fibrous histiocytoma. Two patients with osteosarcoma and the one with malignant fibrous histiocytoma experienced objective partial tumor regression for 1, 3, and 2 months, respectively. Fever, anorexia, myalgia, fatigue, lethargy, and moderate myelosuppression were observed commonly, and some patients developed mild nausea, vomiting, and diarrhea. No patient withdrew because of toxicity and no dose reductions were necessary except adjustments for changes in body surface area secondary to weight loss. [Cancer Treat Rep 71:747-748, 1987]**

Following the recognition during phase I studies of recombinant human leukocyte A interferon that this product has antitumor properties (1), we began a treatment trial in chemotherapy-resistant patients with advanced bone sarcomas. A report from an earlier Memorial Sloan-Kettering Cancer Center trial utilizing natural human leukocyte interferon indicated that in six of eight patients whose metastatic osteosarcomas were associated with increased serum levels of alkaline phosphatase, transient decreases had occurred in the serum concentration of this enzyme (2). Among their 11 patients with osteosarcoma and one with chondrosarcoma none experienced objective tumor regression. The present report describes our phase II results with the pure recombinant product.

## MATERIALS AND METHODS

Between June 30, 1982, and March 14, 1984, 20 patients with metastatic histologically confirmed sarcomas of bony origin were treated with recombinant human alfa interferon (interferon alfa-2a, recombinant; Roche, Roferon-A). All patients had measurable or objectively evaluable disease, expected survival of at least 3 months, and Eastern Cooperative Oncology Group performance status (PS) of 3 or better (PS of 3, one patient; PS of 2, nine; PS of 1, seven; and PS of 0, three). All patients had total leukocyte and platelet counts of at least 3000/$\mu$l and 100,000/$\mu$l, respectively, serum creatinine $< 1.9$ mg/dl, and total serum bilirubin $\leq 1.4$ mg/dl. No significant infections or other malignancies were apparent, and no severe heart disease was present. All patients had recovered from previous surgery, irradiation, and chemotherapy; no patient had previously received any type of interferon treatment. Patients with severe pulmonary insufficiency were excluded as were pregnant or lactating women. All patients were known to be refractory to standard chemotherapy for advanced sarcomas.

Each patient was scheduled to receive thrice-weekly treatment with interferon in im doses of $50 \times 10^6$ IU/m$^2$ for 12 weeks. They were not allowed to receive aspirin or other nonsteroidal anti-inflammatory agents or other antineoplastic treatments during the time in this study. Patients who experienced disease progression at any time were removed from the study. However, those patients with objective tumor regression at the end of the 12-week scheduled treatment were allowed to continue interferon treatment until improvement ceased and disease progressed or had been stable for 2 months.

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Patients with mild or moderate toxicity from interferon were to continue treatment in full doses; however, any more extensive toxic effects were to lead to temporary or permanent withholding of treatment according to our protocol.

Traditional criteria for objective tumor regression, disease progression, and stable disease were utilized in this study. For measurable disease at least a 50% reduction in the sum of the products of longest perpendicular diameters of measured lesions was required to indicate objective regression in the absence of any new lesion. For evaluable disease objective regression was indicated by a definite decrease in tumor size agreed upon by two investigators in the absence of any new lesions. Objective disease progression of measurable disease was indicated by an increase of at least 25% in the sum of the products of longest perpendicular diameters or by the appearance of a new lesion. Similarly, for evaluable disease, progression was identified when two investigators agreed that definite increase in tumor size had occurred. Stability existed when neither regression nor progression criteria were met. All patients were followed regularly until death.

## RESULTS

Among the 20 patients, 17 had osteosarcoma and the three others had fibrosarcoma, mesenchymal chondrosarcoma, and malignant fibrous histiocytoma. Sites of origin were femur, eight patients; tibia, seven; humerus, two; pelvis, two; and calcaneus, one. All patients had received primary surgical treatment and 15 of the patients had undergone at least one thoracotomy. They had also received a median of two (range, one to six) different chemotherapy regimens previously. The patients were from 13 to 62 years of age (median, 25.5) and included 14 men and six women. All patients except two had disease clearly measured in two diameters.

Partial tumor regression was observed in two patients with osteosarcoma and one with malignant fibrous histiocytoma for 1, 3, and 2 months, respectively, with total interferon treatment durations of approximately 4, 6, and 4 months. Three other patients had stable disease, each for 2 months, and interferon was stopped in these patients by the end of the third month of treatment. The other 14 patients experienced tumor progression and stopped treatment within 2 months after beginning interferon without any evidence of disease regression. Among the three patients who experienced objective tumor regression during interferon treatment, none had experienced tumor regression during previous chemotherapy for advanced disease. Two of the three patients achieving disease stability on interferon had previously experienced tumor regression during cytotoxic drug treatment. Only two of the 14 patients whose disease progressed within 2 months after beginning interferon had previously experienced chemotherapy-induced tumor regressions. Thus, no clear relationship seemed apparent between prior chemotherapy responsiveness and current response to interferon in this study. Median time to disease progression for the 20 patients was 46 days (range, 20–154), and median survival time was 173 days (range, 40–682) after entering the study. Among the 11 patients with elevated serum concentrations of alkaline phosphatase only one experienced a decline following interferon treatment.

Leukocyte count nadirs ranged from 1500 to 6800/$\mu$l (median, 2750/$\mu$l) and platelet count nadirs ranged from 64,000 to 421,000/$\mu$l (median, 172,500). Increases of two to three times the normal level of serum transaminase regularly were observed, but in only one patient direct-reacting serum bilirubin increased (to 0.7 mg/dl). No infections or bleeding complications of myelosuppression were observed.

Fatigue, myalgia, fever, anorexia, and lethargy were commonly observed, and several patients experienced mild diarrhea, nausea, and vomiting; however, no patient withdrew or was removed from the study because of drug toxicity and no dose reductions were necessary except adjustments for changes in body surface area due to weight loss.

## DISCUSSION

Although singly not an effective therapy for advanced metastatic osteosarcoma, alfa-2a recombinant human interferon is demonstrably active in a minority of patients. We did not observe the reduction in high serum alkaline phosphatase concentrations previously reported by investigators utilizing natural human leukocyte interferon in a daily schedule at somewhat lower doses (2). Serum alkaline phosphatase levels were normal and remained so in the three patients who experienced objective tumor regression. This modest antisarcoma activity conceivably might be clinically useful as an adjunct to cytotoxic drug regimens effective against osteosarcomas. The dose intensity utilized in this study is very near to the maximum tolerable level for this agent.

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BRIEF REPORTS

# Tamoxifen Therapy in Unresectable Adenocarcinoma of the Pancreas[1]

**Alfred Wong,* Alexander Chan, and Keith Arthur[2]**

**Twenty-four patients with biopsy-documented unresectable ductal adenocarcinoma of the pancreas were treated with tamoxifen. Six of 15 postmenopausal female patients had documented prolonged survival, with three living > 2 years. [Cancer Treat Rep 71:749-750, 1987]**

Estrogen receptors have been found in a variety of human gastrointestinal cancers including colorectal, stomach, and hepatocellular and ductal adenocarcinoma of the pancreas (1,2). However, in very few of these cancers has the potential benefit of endocrine therapy ever been adequately assessed or correlated with receptor content. British (3) and Swedish (4) investigators initiated trials using tamoxifen in patients with unresectable ductal adenocarcinoma of the pancreas. The Swedish group reported a median survival time of 8.5 months, with three of 14 patients surviving for 22 months. These are notable results and merit further investigation. This paper reports a pilot study involving 24 patients with biopsy-documented unresectable ductal adenocarcinoma of the pancreas treated with tamoxifen.

## PATIENTS AND METHODS

Twenty-four consecutive patients with biopsy-documented ductal adenocarcinoma of the pancreas were entered in the study between September 1983 and December 1985. There were 15 postmenopausal women (56–87 years of age) and nine men (51–78 years of age). Twenty patients had a laparotomy for biopsy, bypass procedures, or partial pancreatectomy and four patients had the diagnosis established by ultrasound-guided needle biopsy without laparotomy. Surgery and needle biopsy were performed in local community hospitals prior to referral and entry in the study. With confirmation of tissue diagnosis and known unresectable residual tumor as documented by laparotomy or radiological investigation, treatment was started with tamoxifen at 20 mg orally twice daily. Patients had regular follow-up at 2–3-month intervals until the time of death. Therefore, the endpoint of evaluation is patient survival time from time of diagnosis. Estrogen receptor status is not available in this study since pancreatic cancer tissue is not routinely submitted for assay at the time of laparotomy in local community hospitals.

## RESULTS

The duration of survival from the time of diagnosis for individual patient, age, type of surgical operation, TNM staging, site of metastases, and Eastern Cooperative Oncology Group (ECOG) status are shown in table 1. Up to October 1986, only one woman is alive (24-month follow-up). There are no male survivors. Median survival durations for the female and male subgroups are 10 and 4 months, respectively. The overall median survival for the entire group is 7 months. Six women survived > 1 year; three lived > 2 years.

## DISCUSSION

The grim prognosis of ductal adenocarcinoma of the pancreas is well known. In a review of 61 reported series encompassing 15,000 patients, Gudjonsson et al (5) found a median survival period of 4 months. A similar survival time of 3.3 months was reported in the SEER (Surveillance, Epidemiology, and End Results) data including 5343 patients (6). Patient survival did not vary significantly by sex. Median survival for male and female patients was 3.1 and 3.6 months, respectively. Bender and Carter (7) reviewed the data obtained from 15 literature reports for palliative cholecystojejunostomy or choledochojejunostomy in 1442 patients. The mean survival time was 5.7 months. Therefore, as suggested by Coutsoftides et al (8), the survival time of patients undergoing a palliative bypass procedure is fairly similar to that of untreated pancreatic cancer patients.

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TABLE 1.—Patient characteristics

| Patient No. | Age (yrs) | Survival (mos) | Surgery | TNM staging | Site of metastases | ECOG status |
|---|---|---|---|---|---|---|
| | | | *Females* | | | |
| 1 | 70 | 29 | Laparotomy and biopsy | $T_3N_1M_1$ | Liver | 1 |
| 2 | 71 | 28 | Cholecystojejunostomy | $T_3N_1M_1$ | Peritoneum | 1 |
| 3 | 70 | 16 | Needle biopsy | $T_2N_xM_1$ | Liver | 2 |
| 4 | 60 | 15 | Partial pancreatectomy | $T_1N_1M_0$ | | 0 |
| 5 | 74 | 12 | Choledochoduodenostomy | $T_3N_1M_1$ | Liver | 2 |
| 6 | 63 | 10 | Cholecystojejunostomy | $T_3N_1M_1$ | Liver | 0 |
| 7 | 69 | 10 | Laparotomy and biopsy | $T_3N_1M_1$ | Liver | 1 |
| 8 | 87 | 8 | Choleduodenostomy | $T_3N_1M_1$ | Liver | 2 |
| 9 | 59 | 8 | Choleduodenostomy | $T_3N_1M_1$ | Liver | 1 |
| 10 | 71 | 6 | Laparotomy and biopsy | $T_3N_1M_1$ | Liver | 2 |
| 11 | 59 | 6 | Partial pancreatectomy | $T_1N_1M_0$ | | 0 |
| 12 | 65 | 4 | Laparotomy and biopsy | $T_3N_1M_1$ | Lung | 1 |
| 13 | 60 | 4 | Choledochoduodenostomy | $T_3N_1M_1$ | Liver | 1 |
| 14 | 56 | 3 | Cholecystojejunostomy | $T_3N_1M_1$ | Liver | 1 |
| 15 | 66 | 24 (alive) | Partial pancreatectomy | $T_1N_1M_0$ | | 0 |
| | | | *Males* | | | |
| 1 | 65 | 11 | Cholecystojejunostomy | $T_3N_1M_0$ | | 1 |
| 2 | 51 | 7 | Cholecystojejunostomy | $T_3N_1M_0$ | | 1 |
| 3 | 63 | 5 | Needle biopsy | $T_2N_xM_1$ | Liver | 0 |
| 4 | 61 | 5 | Laparotomy and biopsy | $T_3N_1M_1$ | Liver | 2 |
| 5 | 76 | 5 | Choledochojejunostomy | $T_3N_1M_0$ | | 1 |
| 6 | 61 | 4 | Laparotomy and biopsy | $T_3N_1M_1$ | Peritoneum | 1 |
| 7 | 62 | 3 | Laparotomy and biopsy | $T_3N_1M_1$ | Liver | 2 |
| 8 | 59 | 3 | Needle biopsy | $T_2N_xM_1$ | Liver | 2 |
| 9 | 78 | 2 | Needle biopsy | $T_2N_xM_1$ | Liver | 2 |

In the past, the inability to measure bidimensional tumor response had made it particularly difficult to evaluate the effects of treatment on pancreatic cancer. Therefore, we have chosen the patient's survival time from diagnosis, a clearly defined parameter, to be the endpoint of evaluation in this study. Our study showed an overall median survival of 7 months. This is similar to results reported in a controlled study by Tonnesen and Kamp-Jensen (9). They reported a median survival of 7 months for ten pancreatic cancer patients treated with tamoxifen as compared to a 3-month period in 14 untreated patients. We have observed that all six patients who lived $> 1$ year were postmenopausal women. Three women lived $> 2$ years (range, 24–29 months). The uniformly grim prognosis of pancreatic cancer patients with or without palliative surgery suggests that any significant improvement in survival time may likely be due to the effect of tamoxifen treatment rather than a variation in the natural history of the disease. Likewise, the lack of survival benefit as indicated by the median survival of 4 months for our nine treated male patients is similar to the results of the untreated control patients reported by Tonnesen and Kamp-Jensen, suggesting lack of benefit from tamoxifen therapy in male patients with this disease.

Our preliminary findings suggest that a selected subgroup of patients with unresectable ductal adenocarcinoma of the pancreas may have a prolonged survival benefit on tamoxifen treatment. This potential benefit is confined to postmenopausal women.

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BRIEF REPORTS

# Combination of 6-Mercaptopurine, Vincristine, Methotrexate, and Prednisone in Resistant or Relapsed Acute Nonlymphoid Leukemia[1]

**Giuseppe Leone,* Livio Pagano, Roberto Marra, Raffaele Landolfi, Laura Cudillo, Sergio Storti, Giorgio Mango, Generoso Alfano, and Giuseppe D'Onofrio[2]**

**Twenty-six adults with acute nonlymphoid leukemia (ANLL) relapsed or resistant after first-choice chemotherapy including cytarabine and/or anthracyclines were treated with a modified POMP (6-mercaptopurine, vincristine, methotrexate, and prednisone) combination. Complete remission (CR) was achieved in five of eight resistant patients (62%) and in nine of 18 relapsed patients (50%). Intermittent cycles of chemotherapy including daunorubicin, cytarabine, 6-thioguanine, and etoposide were then administered. In the resistant group CR lasted from 11 to 126 weeks (median, 86), with two patients remaining in CR 126 and 108 weeks after remission induction; in relapsed patients, CR lasted from 9 to 50 weeks (median, 31). [Cancer Treat Rep 71:751-753, 1987]**

Standard regimens including anthracyclines and cytarabine (Ara-C) induce complete response (CR) in 60%–80% of patients with acute nonlymphoid leukemia (ANLL) (1–4). Therapy for resistant or relapsing patients is not standardized and disappointing results are common to most protocols (5–7). Recent studies indicate that high-dose Ara-C is more effective than conventional regimens on relapsed or resistant patients, yet prolonged myelosuppression and extrahematological toxicity limit widespread use of high-dose Ara-C in previously treated patients (8).

The POMP combination [6-mercaptopurine (6-MP), vincristine (VCR), methotrexate (MTX), and prednisone (PDN)] has been used as induction remission therapy before the generalized use of Ara-C, leading to a CR rate ranging from 28% to 60% of patients with prior untreated ANLL (1,9,10). Furthermore, the POMP combination has been used with success and without severe toxicity to consolidate CR obtained with induction treatments including Ara-C and daunorubicin (DNR) (3,11). The aim of our study was to investigate the efficacy of a modified POMP combination (pOMp) in ANLL patients resistant to DNR plus Ara-C induction regimen or relapsed after other treatment not including 6-MP, VCR, and MTX.

## PATIENTS AND TREATMENT

From January 1982 until June 1986, 26 adults with resistant (eight patients) or relapsed (18 patients) ANLL were treated with pOMp: 13 men and 13 women 18–65 years of age; mean, 46; median, 49. In the same period, 34 patients with resistant or relapsed ANLL entered our institution; eight of them were excluded from the protocol because of a Karnofsky score $< 70\%$ and/or hepatic damage. Diagnosis of ANLL was based on morphological examination of bone marrow specimens and on histochemical staining according to French-American-British (FAB) classification (12); doubtful cases were studied by immunophenotyping with monoclonal antibodies. All patients but two had been treated according to the protocols of the Italian Cooperative Group GIMEMA (4). All previous therapies did not include 6-MP, MTX, or VCR; they included DNR (26 of 26 patients), Ara-C (24 of 26), 6-thioguanine (17 of 26), and etoposide (six of 26). Diagnosis of relapse was made when two consecutive bone marrow smears from two different sites showed $> 30\%$ blastic cells. Treatment with the pOMp combination was given as follows: VCR (2 mg iv on Day 1); 6-MP (300 mg/m$^2$ orally on Days 1–5); MTX (7.5 mg/m$^2$ iv on Days 1–5); and PDN (40

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mg/m² on Days 1-5). The same cycle was repeated three times at 10-15-day intervals, according to hematological tolerance. CR was defined according to Cancer and Leukemia Group B criteria (13). Patients who achieved CR were given sequential consolidation therapy as follows: etoposide (150 mg/m² iv on Days 1-3) and Ara-C (150 mg/m² sc on Days 1-3) × 3 at intervals of 3-4 weeks; DNR (45 mg/m² iv on Day 1), Ara-C (100 mg/m² sc on Days 1-5), and 6-thioguanine (100 mg/m² orally on Days 1-5) every 3-4 weeks × 3; and pOMp every 2-3 weeks × 3.

## RESULTS

After one to three courses of the pOMp combination CR was achieved in five of eight patients (62%) with resistant ANLL and in nine of 18 patients (50%) with relapsed ANLL. Nine patients (eight relapsed and one resistant) were unresponsive to the pOMp combination (table 1), and three patients (two resistant and one relapsed) died in induction phase. As shown in table 1, response to pOMp was particularly favorable in the presence of a low neoplastic mass (bone marrow blasts < 45%, peripheral leukopenia with few or absent blastic cells). Considering the FAB classification, the best results were obtained in M3-type leukemia; five of six of the relapsed patients and both of the resistant patients achieved CR. Median duration of CR was 86 weeks in resistant patients and 31 weeks in relapsed patients (table 1). Myelosuppression was moderate, transitory, and well-tolerated in all patients achieving CR. Duration of cytopenia (neutrophil count < 500/mm³ and platelet count < 30,000/mm³) was < 14 days, with a median of 8 days in all responsive patients. Toxicity of the pOMp combination was slight. Nausea and vomiting were rarely present, whereas stomatitis (12 patients) was more frequent. When elevation of hepatic enzymes occurred (11 patients), they usually returned to normal range within 20-30 days after the conclusion of three cycles of therapy and were of no clinical importance. Three patients developed diabetes during pOMp treatment. Severe infections were not observed in responsive patients; two unresponsive patients died from bacteriemic infection in induction phase.

TABLE 1.—Result of induction therapy with pOMp and duration of CR as compared with patient characteristics

| Characteristic | No. of patients | No. of CRs | Median duration of CR (wks) |
|---|---|---|---|
| Patients entered | 26 | 14 (57%) | 42 |
| Sex | | | |
| Male | 13 | 9 | 56 |
| Female | 13 | 5 | 20 |
| Age (yrs) | | | |
| 18-49 | 13 | 9 | 55 |
| 50-67 | 13 | 5 | 25 |
| Status | | | |
| Resistant | 8 | 5 | 67 |
| Relapsed | 18 | 9 | 31 |
| Wbc before pOMp | | | |
| < 10,000 cells/mm³ | 22 | 14 | 42 |
| > 10,000 cells/mm³ | 4 | 0 | |
| Peripheral blasts | | | |
| Absent | 16 | 12 | 38 |
| Present | 10 | 2 | — |
| Bone marrow blasts | | | |
| < 45% | 9 | 5 | 52 |
| > 45% | 17 | 9 | 34 |
| Morphology (FAB) | | | |
| M1 | 2 | 0 | — |
| M2 | 11 | 4 | — |
| M3 | 8 | 7 | 35 |
| M4 | 2 | 2 | — |
| M5 | 3 | 1 | — |

## DISCUSSION

Outcome of resistant or relapsed patients is very poor. Resistant patients generally fail to respond to supplementary cycles of first-line therapy; relapsed patients can again respond but a future relapse occurs within a brief period. Thus, they all are preferably treated with different drugs supposedly non-cross-resistant (1,5-7). The remission rate achieved with our pOMp combination is not worse than that obtained with recently introduced new drugs (5-7). It is better than that reported in other series of resistant patients treated with the POMP combination (1); however, in most of these studies the patients with a history of previous therapy with any of the drugs included in the POMP combination were not excluded from the protocol (10). Moreover, the deaths during induction therapy were exceedingly high (> 50%) as compared with more recent standard therapy (1).

The high response rate of our series could have been favored by the low leukemic mass present in most patients at the start of pOMp treatment: two of eight patients considered resistant according to the criteria of our cooperative group (blast cells > 5% after two courses of DNR plus Ara-C) had had a partial response to first-line therapy and all the relapsed patients were diagnosed and treated very early. The CR duration of relapsed patients was brief, but in resistant patients it was similar to that of the patients responsive to DNR plus Ara-C of the same series (4) and probably was favorably influenced by the intensive postremission treatment comprehensive of many drugs. In relapsed patients, being on or off therapy does not appear to affect the remission rate. This fact together with the high response rate in the resistant patients suggests absence of cross-resistance between pOMp combination and standard drugs employed in ANLL. Our results were particularly encouraging in patients with acute promyelocytic leukemia, where CR was achieved in seven of eight patients. Thus, the pOMp combination is likely to represent an alternative to amsacrine (5) in acute promyelocytic leukemia patients who cannot be further treated with anthracyclines because of resistance or cardiotoxicity.

In conclusion, our pilot study suggests the absence of cross-resistance between DNR plus Ara-C and the pOMp combination and favors its employment to eliminate residual leukemic cells resistant to DNR and Ara-C. Its utilization in relapsed patients is jeopardized by the short remission duration.

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BRIEF REPORTS

# Phase II Study of Continuous-Infusion Diaziquone in Relapsed/Refractory Acute Nonlymphocytic Leukemia[1,2]

**Philip Schulman,[3,*] Roger Davis,[4] Edward Lee,[5] John Ellerton,[6] and Harry Staszweski[7,8]**

***For the Cancer and Leukemia Group B***

**A total of 74 patients with relapsed/refractory acute nonlymphocytic leukemia were entered in a phase II trial of diaziquone (AZQ) administered by continuous infusion at a dose of 28 mg/m$^2$ a day for 5 days. Complete remission (CR) was achieved in 12 of the 67 evaluable patients (18%), all occurring in relapsed patients (12 of 55, 22%). There were eight partial responders (one of whom was in the refractory group). Three of these had normal M-1 bone marrow but did not meet peripheral blood criteria for CR. The median duration of CR was 13.6 weeks (range, 4–40+). Nonhematologic toxicity was mild to moderate and easily manageable. Hematologic toxicity was severe or life threatening in all patients. This study confirms the activity of AZQ when given as a continuous infusion to patients with relapsed acute nonlymphocytic leukemia. [Cancer Treat Rep 71:755–757, 1987]**

Although remission induction in adult acute nonlymphocytic leukemia (ANLL) has remarkably improved over the last 5 years, 20%–30% of patients will fail to achieve an initial complete remission (CR) and 70% of those who achieve a CR will relapse within 2 years (1,2). A small percentage of patients can be reinduced with standard regimens, but usually only for a short duration (3,4). Thus, a pressing need remains for effective new agents.

Diaziquone (AZQ) is a rationally synthesized drug designed to penetrate the CNS. AZQ has activity in vitro against murine leukemia cell lines L1210 and P388. Phase I studies with bolus AZQ showed no activity in ANLL (5). AZQ has a short terminal half-life when administered in a bolus schedule. Because of this, a continuous-infusion regimen was tested in a recent study which reported a 23% CR rate in patients with relapsed ANLL (6). This trial was undertaken at a lower dose because of the activity seen in that trial and the severe myelosuppression encountered.

## MATERIALS AND METHODS

Between December 1984 and May 1986, 74 patients were entered on this trial. Patients with histologically documented ANLL [based on standard French, American, British (FAB) criteria] primarily refractory to treatment, or in first or second relapse, were eligible provided they were 15 years of age, had two prior inductions, had a performance status (PS) $\leq 2$, had a life expectancy of 6 weeks, had had no chemotherapy or radiation therapy in the previous 2 weeks, had normal kidney and liver function, and had signed informed consent consistent with federal regulations and local hospital Human Protection Committees.

The National Cancer Institute supplied AZQ in powdered form with solvent and diluent. AZQ was administered by continuous infusion for 5 days at a dose of 28 mg/m$^2$. Subsequent therapy was determined by Day 15 bone marrow and nonhematologic toxicity. Patients who had residual blasts and cellular bone marrows had dose

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escalation to 28 mg/m$^2$ for 6 days, provided no severe or life-threatening nonhematologic toxicity was encountered. Once CR was achieved, no further treatment was given. Standard Cancer and Leukemia Group B (CALGB) criteria for marrow and hematologic response were used.

## RESULTS

Seventy-four patients were entered on this study. Five patients were ineligible, four because they had received more than two prior induction attempts and one because of a PS of 3. Two patients have not had complete data submitted. Patient characteristics and responses are entered in tables 1 and 2.

This was a representative group of patients with refractory/relapsed ANLL. The age distribution was weighted toward patients > 40 years of age. M-2 and M-4 represented the most common FAB classifications. Most patients had PS ≤ 1. Responses were seen in every age group and FAB classification. CR was seen only in patients who had achieved at least one prior remission (12 of 55 patients, 22%). Duration of CR measured from date of CR to failure (death from any cause or relapse) was a median of 13.6 weeks with a range of 2–45 weeks. The failure at 45 weeks was the only failure beyond 15 weeks, and four patients were last reported in continuing CR at 29, 30, 38, and 43 weeks. One patient who had failed a second induction attempt with cytarabine-daunorubicin had a partial response (PR).

There were eight PRs present. Three of these patients had normal bone marrow (M-1) but did not meet criteria for hematologic remission, because of persistent anemia in one patient and persistent thrombocytopenia in two patients. One patient with leukemic meningitis with no other therapy achieved both a CNS and a bone marrow response.

TABLE 1.—Patient characteristics

| Characteristic | No. of patients (%) |
|---|---|
| Entered | 74 |
| Eligible | 67 |
| Age | |
| < 40 | 16 (24) |
| 40–59 | 20 (30) |
| 60 | 31 (46) |
| Sex | |
| Male | 32 (48) |
| Female | 35 (52) |
| PS | |
| 0–1 | 50 (75) |
| 2 | 17 (25) |
| FAB classification | |
| M-1 | 14 (21) |
| M-2 | 20 (30) |
| M-3 | 4 (6) |
| M-4 | 21 (31) |
| M-5 | 5 (7) |
| M-6 | 2 (3) |
| Not reported | 1 (1) |

TABLE 2.—Response*

| Response | Prior CRs 0 | 1 | 2 | Total |
|---|---|---|---|---|
| CR | 0 | 11 | 1 | 12 |
| PR | 1 | 5 | 2 | 8 |
| Alive | | | | |
| No response | 4 | 17 | 2 | 23 |
| Died | | | | |
| Leukemic marrow | 1 | 10 | 0 | 11 |
| Unknown marrow | 1 | 2 | — | 3 |
| Aplastic marrow | 5 | 4 | 1 | 10 |

*Values = No. of patients.

Myelosuppression was severe or life threatening in all patients. Median time to bone marrow recovery (polymorphonuclear neutrophils > 500) was 37 days (range, 28–70 days). Nonhematologic toxicity was mild or moderate and manageable, in most cases. Mod rate gastrointestinal toxicity was found in 37% of patients, but there was severe or life-threatening diarrhea in 10% of patients possibly related to antibiotics. Thirteen percent of patients had severe pulmonary toxicity primarily related to intercurrent infections. Six patients had skin toxicity. Mild to moderate hepatotoxicity (enzyme elevation) was present in 32%. Moderate alopecia was found in only 25%. Life-threatening or severe renal toxicity was present in 7%, all of whom were on renal-toxic antibiotics.

## DISCUSSION

AZQ given as continuous infusion has activity in patients with relapsed acute nonlymphocytic leukemia. The CR rate in this trial closely corresponds to previous data. There was a somewhat reduced duration of severe myelosuppression compared to the previous continuous-infusion trial which utilized AZQ (6). The current trial had a median time to marrow recovery of 37 days. This is compared to a previous trial which had a median time to marrow recovery of 49 days. This difference is probably due to the reduced dose in the present study.

It is of interest that three patients had normal bone marrow but failed to recover sufficiently in the peripheral blood to qualify as a CR. These data are similar to a previous trial (6). Although these patients are classified as PRs, these appear to be meaningful, as their time to relapse is similar to the patients with CR (median, 9 weeks). Duration of response to AZQ closely approximates previous trials of remission duration in relapsed patients (3,4). The current trial has four patients with response continuing > 30 weeks.

As previously noted, nonhematologic toxicity of AZQ is mild and the drug is very well tolerated. This would make it useful in conjunction with other agents currently useful in relapsed acute myelogenous leukemia (AML). The CALGB is currently undertaking trials of AZQ in conjunction with mitoxantrone, another active drug in relapsed AML (7).

The final place of AZQ in the AML armamentarium remains to be defined. Its lack of nonhematologic toxicity, efficacy, and CNS penetrance (8) make it an attractive agent to be used in untreated patients or possibly as a preparatory agent for bone marrow transplantation.

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BRIEF REPORTS

# Phase II Treatment of Central Nervous System Gliomas With High-Dose Etoposide and Autologous Bone Marrow Transplantation[1]

**Leonard Giannone* and Steven N. Wolff[2]**

**A total of 16 patients with progressive CNS gliomas who had previously received maximal radiation therapy and chemotherapy were treated on a phase II study using high-dose etoposide with autologous bone marrow transplantation to assure hematopoietic recovery. Three patients (19%) responded with both improved neurological status and decreased mass on computerized tomographic scan. Median survival for all treated patients was 4 months with the three responders living 9, 10, and 54+ months. [Cancer Treat Rep 71:759–761, 1987]**

The prognosis for patients with high-grade gliomas of the CNS remains bleak. Long-term disease-free survival is unusual in spite of combined modality therapy consisting of surgery, radiation therapy, and chemotherapy (1). Patients with gliomas which progress following initial therapy have an even poorer prognosis, with a median survival of 7 months (2).

Multiple chemotherapeutic agents have been administered to patients with malignant gliomas. The best results have been obtained with nitrosoureas; however, most responses have been partial and prolongation of survival moderate (3). One method for improving the efficacy of cytotoxic therapy is dose intensification with autologous bone marrow transplantation to ensure hematopoietic recovery (4,5). Previous efforts to use this form of treatment for malignant gliomas have investigated high-dose nitrosoureas. Although preliminary results are encouraging, nonhematologic toxicity is severe (6–8).

Preliminary data evaluating etoposide in the treatment of malignant gliomas indicate that clinical activity is present in spite of low cerebrospinal fluid penetration (9,10). We report here the treatment of 16 patients with recurrent gliomas with high-dose etoposide and autologous bone marrow transplantation.

## MATERIALS AND METHODS

A total of 16 patients were entered into the study between June 1981 and August 1986. Patient characteristics are shown in table 1. Prior to treatment, 13 patients underwent resection of the neoplasm (table 2). In six the resection was intended to be complete. All patients had progressive disease after maximal radiotherapy, and nine after treatment with carmustine-containing chemotherapy. In 11 patients etoposide was given at the time of first progression although five patients were treated when their disease progressed following second-line therapy. Patients were eligible for high-dose etoposide provided they had recovered from previous therapy and demonstrated progressive tumor measurable by computerized tomographic (CT) scan.

Etoposide was administered at a dose of 600–800 mg/m$^2$/day for 3 consecutive days iv (total dose, 1800–2400 mg/m$^2$) followed in 3 days by autologous transplantation of previously cryopreserved bone marrow. For administration of etoposide, one-third of the daily dose (diluted in 500 ml of normal saline) was infused over 1 hour and repeated two times. Patients received a second course of etoposide 6 weeks after the first if tumor progression was not documented. Eight patients were retreated and all received a second infusion of autologous bone marrow.

Marrow harvest, cryopreservation, and reinfusion were performed as previously described (4). The median number of nucleated cells reinfused was $1.3 \times 10^8$/kg (range, $0.5–2.0 \times 10^8$/kg). Patients were treated in single rooms without specialized isolation procedures. Right atrial multilumen catheters, hyperalimentation, broad-spectrum antibiotics, and irradiated (2500 cGy) blood components were used as indicated.

For evaluation, all patients underwent a detailed neurologic examination and contrast-enhanced CT scan of

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TABLE 1.—Characteristics of 16 patients with recurrent CNS gliomas

| Characteristic | No. of patients |
|---|---|
| Median age in yrs (range): 41 (14-54) | |
| Performance status (Karnofsky) | |
| 80%-100% | 8 |
| 70%-80% | 0 |
| 50%-70% | 3 |
| < 50% | 5 |
| Histology | |
| Glioblastoma multiforme | 8 |
| Anaplastic astrocytoma | 3 |
| Astrocytoma—grade I-II | 3 |
| Glioma—high grade | 2 |

TABLE 2.—Previous therapy of 16 patients with recurrent CNS gliomas

| Therapy | No. of patients |
|---|---|
| Original surgical procedure | |
| Partial resection | 7 |
| Total resection | 6 |
| Biopsy only | 3 |
| Second surgical procedure | |
| Partial resection | 3 |
| Total resection | 3 |
| Radiotherapy | 16 |
| Chemotherapy | |
| High-dose carmustine | 5 |
| Standard-dose carmustine | 4 |

the brain immediately before therapy, 1 month after therapy, and periodically as needed. Response was defined in a manner similar to that reported by Levin et al (11) and required an improvement in neurologic findings and a decrease in contrast enhancement on CT scan while the patient was receiving a constant or decreasing dose of glucocorticoids. Survival was measured from the day of marrow transplantation. For response evaluation, treatment-related death was defined as no tumor response.

## RESULTS

Three of 16 patients (19%) experienced a tumor response (95% confidence intervals, 7%–43%). The tumor histology in responding patients consisted of one glioblastoma multiforme, one high-grade glioma, and one low-grade astrocytoma. All responding patients were treated with two cycles of etoposide at the highest dose level. Median survival of the 16 treated patients was 4 months (range, 0–54+) with the three responders living 9, 10, and 54+ months. All nonresponders died of progressive tumor before 8 months.

A total of 24 courses of therapy were evaluated for toxic effects. Nonhematologic toxicity of mild degree occurred in seven courses and consisted of nausea, vomiting, and mucositis. Hematologic toxicity was severe with all patients having an absolute neutrophil count $< 500/mm^3$ for a median of 7 days (range, 5–14). Eighteen courses required platelet transfusion to maintain a platelet count $> 50,000/mm^3$. Fever necessitating broad-spectrum antibiotics occurred in 20 courses and two patients died of uncontrolled infection during the period of severe neutropenia. Toxicity was not clearly greater during the second course of therapy.

## DISCUSSION

The chemotherapy of malignant gliomas is currently unsatisfactory. Modest improvement in survival is seen with some agents such as the nitrosoureas, but complete regression of mass lesions and prolonged disease-free survival are rare (1). In an effort to deliver an increased dose of etoposide to the tumor, we treated 16 patients with high-dose iv etoposide followed by autologous bone marrow transplantation. The marrow was transplanted to limit the duration of myelosuppression although etoposide is clearly not marrow ablative. All patients had progressive disease after prior maximal radiotherapy. Thirteen patients had undergone surgical resection and nine had received carmustine-containing chemotherapy prior to high-dose etoposide. Three patients responded, living 9, 10, and 54+ months. The median survival of all treated patients was 4 months. Hematologic toxicity was severe and mandated in-hospital management since all patients experienced severe myelosuppression. Two patients developed fatal infectious complications during the period of severe neutropenia.

Our treatment results indicate an effectiveness rate of 15% which is not dissimilar to the experience using standard-dose etoposide (9,12). Tirelli et al (9) observed three responses in 18 patients treated with etoposide at conventional doses (200 $mg/m^2$). A previous attempt to administer high-dose etoposide (600–1000 $mg/m^2$) resulted in one partial response in 24 patients (10). Compared to these results, carmustine in standard or high dose appears superior (3,6). At present, as a single agent, high-dose etoposide is not to be recommended in the treatment of malignant glioma due to its modest activity and severe myelosuppressive consequences. Further evaluation of this agent in standard or high dose will require the demonstration of synergism with other cytotoxic agents.

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# Phase II Trial of Oral Piritrexim (BW301U) in Patients With Stage III Non-Small Cell Lung Cancer[1,2,3]

**Mark G. Kris,* Richard J. Gralla, M. Thomasine Burke, Lloyd D. Berkowitz, Linda D. Marks, David P. Kelsen, and Robert T. Heelan[4,5]**

Piritrexim (BW301U; 2,4-diamino-6-(2,5-dimethoxybenzyl)-5-methylpyrido [2,3-*d*] pyrimidine) is a lipid-soluble antifol. It is a potent inhibitor of mammalian dihydrofolate reductase which produces in vitro and in vivo antitumor effects (1-3). The cytotoxic effects of piritrexim were only partially reversed by leucovorin calcium. Unlike other lipid-soluble antifolate compounds that can produce central nervous system toxicity due to inhibition of histamine *N*-methyl transferase, piritrexim demonstrates only weak inhibition of this enzyme (1). In dogs given this drug (10 mg/kg) orally, the average absolute bioavailability was 0.64. In a phase I trial of oral piritrexim in adult patients with solid tumors, leukopenia, thrombocytopenia, rash, and mucositis were observed (4). Based on these data, a phase II trial of oral piritrexim was undertaken in individuals with advanced non-small cell lung cancer.

## PATIENTS AND METHODS

Thirty-five patients with stage III non-small cell lung cancer were entered in this study. Eligibility criteria included the presence of nonirradiated measurable or evaluable indicator lesions; Karnofsky performance status $\geq 50\%$; normal hepatic, renal, and cardiac functions; leukocyte count $\geq 4000/\mu l$; and platelet count $\geq 120{,}000/\mu l$. Piritrexim was administered at a dose of 160 mg/m$^2$, orally, twice daily for 5 days (ten doses total). Because only 100-mg capsules were available, each calculated dose was rounded down to the nearest 100 mg. Doses in subsequent courses were increased by 25% if the maximum toxicity was 0 or 1+ in the prior course; it was decreased by 25% or 50%, respectively, if 3+ or 4+ toxicity was observed. Standard response and toxicity criteria were used (5).

## RESULTS

Characteristics of the patients entered into the study are shown in table 1. Thirty-three of the 35 patients (94%) received an adequate trial. One inadequately treated patient had no change in indicator lesion and only 1+ toxicity during a single course. The other patient did not have a follow-up x-ray. None of the patients previously treated with chemotherapy had received antifolate compounds. A median of three courses (range, one to 12) of piritrexim were given. One previously untreated woman with an evaluable indicator lesion experienced a major objective response (5) lasting 4 months. All other patients had no change or disease progression.

### Toxicity

Stomatitis, rash, leukopenia, thrombocytopenia, nausea, and vomiting were the most common side effects. The maximum toxicity grades for each effect are shown in table 2. The wbc count nadir was noted on Day 8, and the platelet count nadir was noted on Day 15. Grade 1 or 2 diarrhea, alopecia, hematuria, anorexia, and fatigue were also observed. The maximum toxicity grade noted in individual patients was 1+ in 10%, 2+ in 42%, 3+ in 42%, and 4+ in 6%. Two-thirds of the patients studied experienced at least two different Grade 2, 3, or 4 toxic effects. There were no treatment-related deaths.

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[3] Presented in part at the 77th Annual Meeting of the American Association for Cancer Research, Los Angeles, California, May 7-10, 1986.

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[5] We thank Dr. Lee Schwartzberg, Dr. James O'Brien, Dr. Janice Kirsch, Ms. Elizabeth West, Ms. Irene Cibas, and Mr. Al Guasparic for their assistance.

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TABLE 1.—Patient characteristics and response

| | All patients | Prior chemotherapy | No prior chemotherapy |
|---|---|---|---|
| No. of patients | | | |
| Entered | 35 | 19 | 16 |
| Adequate | 33 | 19 | 14 |
| Median age (yrs) | 63 | 58 | 66 |
| Sex: male/female (%) | 52/48 | 47/53 | 57/43 |
| Karnofsky performance status (%) | | | |
| 80%–90% | 27 | 32 | 21 |
| 50%–70% | 73 | 68 | 79 |
| Cell type (%) | | | |
| Adenocarcinoma | 64 | 58 | 72 |
| Epidermoid carcinoma | 24 | 32 | 14 |
| Large cell carcinoma | 12 | 10 | 14 |
| Stage III: M0/M1 (%) | 9/91 | 14/86 | 5/95 |
| Bone metastases (%) | 30 | 11 | 57 |
| Prior radiotherapy (%) | 36 | 42 | 29 |
| Major response rate (%) | 3 | 0 | 7 |
| 95% confidence limits for predicted response rate (%) | 0.3–15 | 0–15 | 0.4–31 |

TABLE 2.—Toxicity

| | % of all patients ($N = 33$) with maximum toxicity Grade—* | | | | |
|---|---|---|---|---|---|
| Toxic effect | 0 | 1+ | 2+ | 3+ | 4+ |
| Stomatitis | 15 | 22 | 33 | 30 | 0 |
| Rash | 27 | 40 | 30 | 3 | 0 |
| Leukopenia | 30 | 22 | 30 | 15 | 3 |
| Thrombocytopenia | 43 | 22 | 19 | 13 | 3 |
| Nausea/vomiting | 54 | 12 | 34 | 0 | 0 |

*World Health Organization scale (5).

## DISCUSSION

With only one of 33 adequately treated patients experiencing a major response, the predicted true response rate for piritrexim is $< 15\%$ ($P = 0.05$). The observed major response rate for patients previously treated with chemotherapy was 0 (95% confidence limits for the predicted true response rate, 0–15%). For individuals who had not received prior chemotherapy, the observed major response rate was 7% (95% confidence limits, 0.4%–31%). Piritrexim, in the dose and schedule used, had only marginal activity in this group of patients with non-small cell lung cancer.

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# Phase II Study of Esorubicin in Patients With Advanced Colorectal Carcinoma and No Prior Chemotherapy: An Illinois Cancer Council Trial[1,2]

**Gershon Y. Locker,[3,*] Thomas Hoeltgen,[4] Lary Kilton,[5] Stuart Krauss,[6] Mark McNamara,[3] Richard Blough,[7] and Carole Johnson[7]**

Esorubicin (DxDx), an anthracycline antibiotic derivative of doxorubicin with a similar mechanism of action, was synthesized with the aim of developing an active but less cardiotoxic drug (1). It is very effective against several murine tumors (2) and human colon cancer transplanted into athymic mice (3). The Illinois Cancer Council has completed a phase II study of DxDx in patients with colorectal carcinoma to define its activity in that disease.

## MATERIALS AND METHODS

Patients were eligible if they had histologically proven recurrent or metastatic, measurable colorectal carcinoma. All patients had an Eastern Cooperative Oncology Group (ECOG) performance status of 0–2; acceptable hematologic, renal, and hepatic parameters; no prior heart disease; an expected survival of 60 days; and no previous chemotherapy. DxDx was administered at a dose of 30 mg/m$^2$ iv every 3 weeks (4), with subsequent dose adjustment to achieve a nadir wbc count of 2000–3000/mm$^3$. Patients with prior radiation therapy were begun at a dose of 25 mg/m$^2$. A pretreatment gaited cardiac pool scan or echocardiogram was optional; it was mandatory in all patients at a cumulative dose of 200 mg/m$^2$ and every third treatment thereafter. Objective response was as defined by ECOG criteria.

## RESULTS

Pretreatment characteristics of the 25 patients entered in the trial are summarized in table 1. All patients were evaluable for toxicity and 23 patients who received at least two doses were evaluable for response (two patients received only one dose). As expected, given the design of the study, leukopenia was common (84%); it was severe in ten patients (40%). Thrombocytopenia was rare (8%). Nine patients (36%) had phlebitis or venous spasm associated with pain at the site of injection. Two patients had a substantial drop in cardiac ejection fraction without clinical congestive heart failure; one pa-

TABLE 1.—Patient characteristics

| Characteristic | No. of patients (%) |
|---|---|
| Total | 25 |
| Median age in yrs (range): 64 (43–73) | |
| Male | 13 (52) |
| Female | 12 (48) |
| ECOG performance status | |
| 0 | 16 (64) |
| 1 | 6 (24) |
| 2 | 3 (12) |
| Evaluable for toxicity | 25 |
| Leukopenia (cells/mm$^3$) | 21 (84) |
| Wbc count nadir | |
| 3000–4499 | 4 |
| 2000–2999 | 7 |
| 1000–1999 | 7 |
| < 1000 | 3 |
| Thrombocytopenia (cells/mm$^3$) | 2 (8) |
| Platelet count nadir | |
| 90,000–130,000 | 0 |
| 50,000–89,000 | 1 |
| 25,000–49,000 | 1 |
| < 25,000 | 0 |
| Anemia | 17 (68) |
| Nonhematologic toxicity | 18 (72) |
| Phlebitis/venous spasm | 9 |
| Nausea/vomiting | 8 |
| Alopecia | 4 |
| Diarrhea | 3 |
| Stomatitis | 3 |
| Cardiotoxicity | 2 |
| No toxicity reported | 2 (8) |
| Evaluable for response | 23 (92) |
| Partial response | 3 (13) |

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[2] Supported by Public Health Service grant CA-21742 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.
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tient, a partial responder, had received 31 courses (1238 mg/m$^2$), and the other had received five courses (183 mg/m$^2$).

A partial response was seen in three of 23 evaluable patients (13%; 95% confidence levels, 3%–34%), lasting 14–30 weeks. If the two patients who received only one dose are included, the response rate is 12%. Responses were seen in two patients with liver metastases and in one with lung metastases. Median survival of all 25 patients in the study was 35 weeks from entry.

## DISCUSSION

Using the standard criteria of response rate and survival of all patients treated, DxDx has minimal activity against colorectal carcinoma as a single agent. However, the 95% confidence limits for the true response rate do overlap the accepted activity of 5-fluorouracil in this disease. The presence of cardiotoxicity in two patients in a small study where the median cumulative dose received was 78 mg/m$^2$, and only nine patients received $>$ 150 mg/m$^2$, underscores the previously reported high risk of cardiac effect of the drug (5). DxDx has a limited role in the treatment of advanced colorectal carcinoma.

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# Phase II Trial of Esorubicin in the Treatment of Metastatic Carcinoma of the Kidney: A Study of the Northern California Oncology Group[1,2]

**Robert W. Carlson,[3,4,*] Richard D. Williams,[5] Margaret E. Billingham,[3] Marsha Kohler,[4] and Frank M. Torti[3,6]**

Esorubicin is a new anthracycline analog developed by Farmitalia Carbo Erba that differs from doxorubicin in the removal of an oxygen atom from the 4′ position of the amino-sugar moiety. Results reported from clonogenic assay systems suggested that esorubicin might be active against renal carcinoma (1). Further testing in animals documented minimal cardiac toxicity produced by esorubicin (2). The Northern California Oncology Group thus initiated a phase II study of single-agent esorubicin in patients with renal carcinoma.

This phase II trial was initiated in July 1984 and closed to accrual in November 1985. Eligible patients had histologically confirmed metastatic renal carcinoma previously untreated with chemotherapy; Karnofsky performance status $\geq$ 50%; estimated survival $\geq$ 8 weeks; measurable disease; adequate hepatic, renal, and bone marrow functions; and no radiotherapy, immunotherapy, or hormonal therapy for at least 4 weeks. Patients were ineligible if they had a history of serious cardiac disease, concurrent complications such as infection, an active second tumor, or CNS metastasis. Signed informed consent was required.

Treatment with esorubicin at 30 mg/m$^2$ iv was administered every 3 weeks. The dose of esorubicin was modified according to the following criteria: wbc count $\geq$ 3500/mm$^3$ and platelet count $\geq$ 150,000/mm$^3$, 100% dose; wbc count 3000–3499/mm$^3$ or platelet count 100,000–149,999/mm$^3$, 75% dose; and wbc count $<$ 3000/mm$^3$ or platelet count $<$ 100,000/mm$^3$, delay therapy 1 week and recheck blood cell counts.

## RESULTS

Patient characteristics are shown in table 1. The one ineligible patient had pre-existent heart disease and received no drug. Despite the limited prior therapy that the patients had received, no objective responses were observed in the 24 evaluable patients treated in this study, rejecting a response rate of $\geq$ 12% with 95% confidence. Median survival was 11.4 months, and no patient has survived $>$ 21 months.

Common acute toxic effects are also shown in table 1.

TABLE 1.—Patient characteristics and toxicity

| Characteristic | No. of patients (%) |
|---|---|
| Entered | 25 |
| Eligible | 24 |
| Median age in yrs (range): 58 (23–76) | |
| Median Karnofsky performance status (range): 80% (50%–100%) | |
| Prior therapy | |
| Hormonal therapy | 8 (33) |
| Biologic response modifiers | 5 (21) |
| Radiotherapy | 0 |
| Chemotherapy | 0 |
| Toxicity | |
| Lowest wbc count (cells/mm$^3$) | |
| 3000–4499 | 2 (8) |
| 2000–2999 | 7 (29) |
| 1000–1999 | 6 (25) |
| $<$ 1000 | 3 (13) |
| Lowest platelet count (cells/mm$^3$) | |
| 90,000–129,000 | 6 (25) |
| 50,000–89,000 | 4 (17) |
| 25,000–49,000 | 0 |
| $<$ 25,000 | 1 (4) |
| Nausea and vomiting | |
| Mild (Grade 1) | 6 (25) |
| Moderate (Grade 2) | 9 (38) |
| Fever | 3 (13) |

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Myelosuppression and nausea and vomiting were the most common acute toxic effects. No patient experienced clinical congestive heart failure, although endomyocardial biopsy changes (3) consistent with anthracycline injury were observed in the biopsied patients. One patient experienced recurrent ventricular tachycardia felt secondary to an Infusaport catheter that resolved with repositioning of the catheter.

We conclude that esorubicin is an inactive agent in the treatment of metastatic renal cancer.

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# Phase II Study of the Combination of Vinblastine Plus Cisplatin Administered by Continuous 120-Hour Infusion for Patients With Advanced Malignant Melanoma[1]

**E. T. Creagan,* D. L. Ahmann, A. J. Schutt, and D. J. Schaid[2]**

Meaningful systemic strategies for disseminated malignant melanoma (DMM) have been discouragingly elusive. The combination of vinblastine and cisplatin (CDDP) appeared to offer an intriguing therapeutic alternative. Each agent has documented activity against melanoma and utilizes unique antiproliferative mechanisms (1,2). Although prior studies of CDDP-based polychemotherapy for DMM were of marginal benefit, CDDP was typically administered over several hours on a 3–4-week schedule (3). Emerging data have indicated a possible dose-response relationship for CDDP (4). Furthermore, early clinical trials (5) and experimental observations (6) have documented the feasibility and rationale for continuous CDDP infusion. We report here a phase II trial of the combination of vinblastine plus prolonged-infusion cisplatin in patients with DMM.

Twenty-six ambulatory patients with biopsy-confirmed DMM and satisfactory hematologic (wbc count $\geq 4100/mm^3$; platelet count $\geq 130{,}000/mm^3$), renal (serum creatinine $\leq 2.0$ mg/dl), and hepatic (no elevation of direct serum bilirubin) functions participated in the study (table 1). The initial monthly iv doses were vinblastine at 5 $mg/m^2$ on Days 1 and 2 by rapid injection and CDDP at 25 $mg/m^2$ on Days 1–5 by 24-hour infusion in 3000 ml of normal saline.

Prior to initial treatment and subsequent monthly courses, patients underwent a complete medical evaluation including chest x-ray and routine hematologic and chemical tests.

We defined an objective regression as a decrease of $\geq$ 50% in the sum of the products of the diameters of the most clearly measurable indicator lesions without new lesions or symptomatic deterioration. We defined progressive disease as a $>$ 25% increase in any indicator lesion, significant clinical deterioration, or appearance of new lesions. Time to disease progression was calculated from the date of study entry to the date of progression or last follow-up. Duration of response was measured from the date of study entry to the date of progression following regression.

TABLE 1.—Patient characteristics

| Characteristic | No. of patients |
|---|---|
| Total | 26 |
| Median age (yrs): 52 | |
| Males/females | 16/10 |
| Performance score* | |
| 0 or 1 | 20 |
| 2 or 3 | 6 |
| Dominant metastatic site | |
| Visceral | 20 |
| Nonvisceral | 6 |
| Prior systemic chemotherapy | 20 |

*Eastern Cooperative Oncology Group performance score: 0 (fully active) to 4 (totally disabled).

We observed two objective regressions among 26 patients (8% response rate; 95% confidence interval, 1%–25%). A 51-year-old man without prior chemotherapy had a $>$ 50% decrease in a pulmonary nodule and in a left thoracic pleural-based mass for 6.4 months. A 34-year-old man with prior therapy manifested an objective response of a lung lesion for 5 months. Of the 26 patients, 62% were immediate treatment failures. The median (Kaplan-Meier) times to failure and survival were 1.3 and 8 months, respectively.

Most patients had moderate to severe nausea (50%), vomiting (31%), and alopecia (46%). The median leukocyte count nadir was $2.0 \times 10^3$ cells/$mm^3$ (range, 0.5–10.9), and the median platelet count nadir was $188 \times 10^3$ cells/$mm^3$ (range, 14–386). We did not detect any evidence of nephrotoxicity. Two patients developed increasingly disabling sensorimotor peripheral neuropathy after five treatment cycles. This symptom gradually resolved over several months after treatment was discontinued.

We were unable to demonstrate any substantial clinical benefit from this program among these 26 patients with disseminated malignant melanoma. Despite ap-

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parently sound theoretical concepts for a prolonged CDDP infusion, our results would not warrant further investigations. Moreover, the onset of severe peripheral neuropathy would certainly have precluded prolonged treatment.

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# Phase II Study of Iv Menogaril in Patients With Malignant Melanoma: A National Cancer Institute of Canada Clinical Trials Group Study[1,2]

**Peter B. McCulloch,[3] Elizabeth A. Eisenhauer,[4,*] Henry R. Shibata,[5] and Vince P. Young[6,7]**

Menogaril is a semisynthetic anthracycline derived from the antitumor antibiotic nogalamycin. It has demonstrated activity against a number of murine tumors, and some data suggest it retains activity after oral dosing (1,2). Toxic effects in phase I iv studies have included myelosuppression (dose-limiting), concentration-dependent phlebitis, nausea and vomiting, and mild hair loss (3,4). As part of an ongoing effort to test new agents in malignant melanoma, the National Cancer Institute of Canada Clinical Trials Group conducted a phase II study of iv menogaril in previously untreated patients with metastatic melanoma.

## MATERIALS AND METHODS

Patient eligibility criteria included the following: measurable metastatic melanoma; no prior chemotherapy; platelet count $\geq 125 \times 10^9$ cells/L, granulocyte count $\geq 2.0 \times 10^9$ cells/L; normal bilirubin and creatinine; Eastern Cooperative Oncology Group (ECOG) performance status of 0, 1, or 2; left ventricular ejection fraction $\geq 0.5$; and written informed consent. Menogaril was given iv over 2 hours at a starting dose of 200 mg/m$^2$ in 500 ml of 5% dextrose in water every 4 weeks. Doses were escalated to achieve a granulocyte count nadir between 0.75 and $1.5 \times 10^9$ cells/L. History, physical examination, and tumor measurements were repeated every 4 weeks. Response criteria were as follows: *complete response*—the disappearance of all evidence of tumor for a minimum of 4 weeks; *partial response*—a $\geq 50\%$ decrease in the sum of the products of measured lesions for a minimum of 4 weeks; *stable disease*—an increase in tumor of $< 25\%$ or a decrease of tumor of $< 50\%$ for at least 8 weeks; and *progressive disease*—a 25% increase in the size of measured lesions or the appearance of significant new lesions.

## RESULTS

Twenty-one patients were entered in the study. Two patients were ineligible (one due to ECOG performance status of 3; one due to ineligible pathology) and 19 were evaluable for response and toxicity. Patient characteristics and toxicity are shown in tables 1 and 2. Neutropenia of $< 1.0 \times 10^9$ cells/L was seen in ten of 19 cycles at

TABLE 1.—Patient characteristics*

| Characteristic | No. of patients |
|---|---|
| Median age in yrs (range): 49 (30-77) | |
| Sex | |
| Male/female | 10/9 |
| Performance status (ECOG) | |
| 0 | 9 |
| 1 | 9 |
| 2 | 1 |
| Metastatic sites | |
| Liver | 9 |
| Lung | 10 |
| Soft tissue | 12 |
| Bone | 3 |
| Response | |
| Complete | 0 |
| Partial | 0 |
| Stable disease | 4† |
| Progressive disease | 15 |

*$N = 19$ evaluable patients.
†12 wks each.

[1] Received Mar 2, 1987; revised Apr 1, 1987; accepted Apr 3, 1987.
[2] Supported by grants from the National Cancer Institute of Canada and The Upjohn Company of Canada.
[3] Henderson General Hospital, Hamilton, Ontario, Canada.
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[7] We thank the following individuals who, in addition to the authors, contributed patients to the study: Drs. G. Boos and P. Major, Montreal General Hospital, Canada; Drs. R. Lohmann, A. Smith, and J. Skillings, London Regional Cancer Centre, Canada; Dr. A. Loutfi, Royal Victoria Hospital, Montreal, Canada; and Dr. J. Gowing, Hamilton Regional Cancer Centre, Canada.
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TABLE 2.—Toxicity

| Toxic effect | |
|---|---|
| No. of patients | |
| Nonhematologic toxicity | |
| Nausea/vomiting | |
| Mild | 4 |
| Moderate | 5 |
| Phlebitis/sore arm | |
| Mild | 6 |
| Moderate | 3 |
| No. of cycles | |
| Hematologic toxicity* | |
| Nadir neutrophil count ($\times 10^9$ cells/L) | |
| $< 0.5$ | 1 |
| 0.5–0.99 | 9 |
| 1.0–1.49 | 3 |
| 1.5–1.99 | 2 |
| $> 2.0$ | 4 |
| Nadir platelet count ($\times 10^9$ cells/L) | |
| $< 25$ | 0 |
| 25–49 | 0 |
| 50–99 | 0 |
| 100–149 | 1 |
| $> 150$ | 18 |

*200 mg/m$^2$.

200 mg/m$^2$ but no significant thrombocytopenia was documented. Mild or moderate nausea and vomiting was seen in nine patients and mild hair loss in three. Half the patients experienced local toxicity of one or more types: mild soreness at the injection site, true phlebitis, and generalized aching in the arm into which the drug had been infused. No responses were seen. Four patients had stable disease and the remaining 15 progressed on therapy.

## DISCUSSION

The observation of no responses among 19 patients excludes a true response rate of $\geq 15\%$ with 95% confidence. While toxicity was generally mild, the problem of local pain made menogaril difficult to use. No patient in this study had a central venous line inserted, but the use of such lines may be necessary if the drug is given for more than one or two courses. Alternatively, results of an oral phase I trial suggest that oral administration may prove feasible (5). In summary, we conclude that iv menogaril is inactive in malignant melanoma and that the problem of local drug toxicity adds practical difficulties to its use.

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# Phase II Evaluation of Aclarubicin in Refractory Multiple Myeloma: A Southeastern Cancer Study Group Trial[1,2]

**Jon P. Gockerman,[3,*] Harold Silberman,[3] and Alfred A. Bartolucci[4]**

Aclarubicin is an anthracycline derivative being evaluated in hopes of finding an improved therapeutic index over doxorubicin (1–3). The present study was undertaken to evaluate the therapeutic efficacy of aclarubicin in refractory multiple myeloma using an every-3-week schedule.

## MATERIALS AND METHODS

Patients eligible for this study had a confirmed diagnosis of multiple myeloma based on standard criteria and had measurable protein abnormality or measurable plasmacytoma (4). They had been previously treated with standard regimens, at least 4 weeks had elapsed since previous chemotherapy, and there was evidence of disease progression. Karnofsky performance status was $\geq$ 50%. Patients with cytopenias due to their myeloma were eligible. Written informed consent was required. Patients were stratified according to good- and poor-risk groups based on hemoglobin (> 9 g/100 ml), BUN (> 30 mg/100 ml), and calcium (< 12 mg/100 ml) for the good-risk group (5). Patients not previously treated with anthracyclines were preferred. Patients were evaluated for survival, toxic effects, and response if they received one dose of aclarubicin. The starting dose was 100 mg/m$^2$ given as an iv push over 5 minutes once every 3 weeks. If the patients had cytopenia to start (wbc count < 4500/mm$^3$; absolute granulocyte count < 1900/mm$^3$; or platelet count < 130,000/mm$^3$), they were treated with 85 mg/m$^2$ every 3 weeks. The abnormal protein component or plasmacytoma was followed to measure disease response using standard criteria (5). Patients judged as stable or responders were to continue for a maximum of 1 year, whereas patients with disease progression were taken off study.

## RESULTS

Patient characteristics, toxic effects, and treatment results are shown in table 1. There were 16 poor-risk patients and 19 good-risk patients evaluable. There were two patients in the good-risk group with inadequate data for evaluation. The number of courses of aclarubicin was similar between the good- and poor-risk patient groups. Serious granulocytopenia, thrombocytopenia, and nausea and vomiting were seen in both groups as a result of the aclarubicin treatment. The elevation in alkaline phosphatase and serum creatinine seen was felt to be due to disease progression rather than a toxic effect of the drug. No cardiac toxicity was seen.

There were no responses seen in any of the 35 patients treated. Ten patients had no change in their M-spike or plasmacytoma for at least 4 weeks and were considered to have no disease progression (5). Thus, aclarubicin as a single agent in this dose and schedule is not helpful in patients with multiple myeloma who have been heavily pretreated.

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TABLE 1.—Patient characteristics, toxic effects, and treatment results*

| Characteristic | Group | |
|---|---|---|
| | Poor-risk | Good-risk |
| Evaluable | 16 | 19 |
| Inevaluable | 0 | 2 |
| Males/females | 9/7 | 12/7 |
| Median age (yrs) | 63.5 | 60 |
| Karnofsky performance status (%) | | |
| 50–60 | 1 | 1 |
| 70–80 | 8 | 11 |
| 90–100 | 2 | 1 |
| Unknown | 5 | 6 |
| No. of aclarubicin courses given | | |
| 1 | 5 | 5 |
| 2 | 6 | 6 |
| 3 | 1 | 3 |
| 4 | 4 | 4 |
| 5 | — | 1 |
| Duration of treatment (wks) | | |
| 3 | 3 | 4 |
| 6 | 7 | 7 |
| 9 | 2 | 4 |
| 12 | 4 | 3 |
| 15 | — | 1 |
| Toxic effects (grade III and IV) | | |
| Leukopenia | 9 | 7 |
| Neutropenia | 7 | 9 |
| Thrombocytopenia | 7 | 3 |
| Nausea/vomiting | 7 | 10 |
| Creatinine increase | 4 | 4 |
| Alkaline phosphatase increase | 3 | 2 |
| Response (time to progression in mos) | | |
| Stable disease | 5 (1, 1, 3.6, 2.7, 8.1) | 5 (1.6, 2.7, 3.2, 4.1, 9.2) |
| Disease progression | 10 | 14 |
| Died on study | 1 | — |
| Prior treatment† | | |
| MP | 1 | 2 |
| MP, Cy, Adr, Vcr | 3 | 4 |
| MP, BCyP | 1 | 2 |
| MP, BCyP, Cy, Vcr | 5 | 5 |
| BCyP | 3 | |
| MP, BCyP, Cy, Adr, Vcr, Hxm | 1 | 1 |
| No data | 2 | 4 |

*Unless otherwise specified, values = No. of patients.

†M = melphalan; P = prednisone; Cy = cyclophosphamide; Adr = doxorubicin; Vcr = vincristine; B = carmustine; and Hxm = hexamethylmelamine.

# Phase II Study of Oral Idarubicin in Patients With AIDS-Associated Kaposi's Sarcoma[1,2]

**Abraham Chachoua,* Michael Green, Linda Laubenstein, James Wernz, and Franco M. Muggia[3,4]**

Anthracyclines have demonstrable activity when given in combination with other cytotoxic drugs in patients with Kaposi's sarcoma (1,2). This phase II study was initiated in order to define the activity of idarubicin, an analog of daunorubicin, in Kaposi's sarcoma associated with the acquired immune deficiency syndrome (AIDS-KS).

## METHODOLOGY

A total of 15 patients were entered on study (table 1). All patients had histopathologically verified AIDS-related Kaposi's sarcoma with measurable lesions, and were staged according to the previously published New York University classification for AIDS-KS (3).

Other inclusion critera were granulocyte count $\geq$ 1500/dl, platelet count $\geq$ 100,000/dl, bilirubin $\leq$ 1.5 mg/dl, and SGOT and SGPT $\leq$ 1.5 times normal.

The starting dose was 45 mg/m$^2$ orally every 3 weeks. The median number of treatment cycles given was four. Only three patients received $>$ 200 mg/m$^2$ of idarubicin. Of these, one patient received 14 treatment cycles with a cumulative dose of 562 mg/m$^2$.

Traditional criteria for objective tumor regression, disease progression, and disease stability were utilized in this study.

Idarubicin was supplied in 5-mg and 10-mg capsules, and was provided by Adria Laboratories.

## RESULTS

There were no complete or partial responses observed in this study. Median survival was 28 weeks. Flattening and change in color of Kaposi's lesions were seen in one patient on study; however, this was not associated with a change in the two-dimensional measurements of his lesions. A decrease in lymph node size was seen in one other patient, also with no change in cutaneous lesions.

Toxicity was mainly hematologic. Leukopenia was more frequent than thrombocytopenia (table 1). Non-hematologic toxicity was not severe and consisted of

TABLE 1.—Patient characteristics

| Characteristic | No. of patients |
|---|---|
| Entered | 15 |
| Median age in yrs (range): 38 (31-52) | |
| Sex | |
| Male | 15 |
| Female | 0 |
| Median ECOG performance status*: 1 | |
| Stage | |
| I | 0 |
| II | 1 |
| III | 6 |
| IV | 8 |
| Prior AIDS manifestations | |
| *Pneumocystis carinii* pneumonia | 1 |
| None | 14 |
| Prior therapy | |
| Prior chemotherapy | 11 |
| Anthracyclines | 4 |
| Prior radiotherapy | 3 |
| Prior immunotherapy | 5 |
| No. of cycles received | |
| 1 | 2 |
| 2 | 5 |
| 3 | 3 |
| 4 | 5 |
| Toxic effects | |
| Wbc count nadirs ($\times$ 1000/mm$^3$) | |
| 2.0-2.9 | 7 |
| 1.0-1.9 | 5 |
| $<$ 1.0 | 1 |
| Nausea and vomiting | |
| Grade 1 + 2 | 10 |
| Grade 3 | 0 |
| Grade 4 | 0 |
| Diarrhea | |
| Grade 1 | 0 |
| Grade 2 | 4 |
| Grade 3 | 1 |
| Stomatitis | 0 |
| Responses | |
| Complete response + partial response | 0/15 |

*ECOG = Eastern Cooperative Oncology Group.

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[2] Supported in part by a grant from Adria Laboratories.
[3] Rita and Stanley Kaplan Cancer Center, New York University School of Medicine, New York, NY.
[4] We thank Ms. Sharon Dynes for her secretarial assistance.
**Reprint requests to:* Abraham Chachoua, MD, Rita and Stanley Kaplan Cancer Center, New York University School of Medicine, 550 First Ave, New York, NY 10016.

nausea, vomiting, and diarrhea. One patient received a cumulative dose of idarubicin of 562 mg/m$^2$. Follow-up cardiac function evaluation showed no decline in his ejection fraction or clinical evidence of cardiac dysfunction. Three patients developed pneumocystis pneumonia while on study.

## DISCUSSION

The results indicate that, in this patient population with this dose and schedule, idarubicin is not active in the treatment of AIDS-KS. The lack of responses in 15 patients ensures with 95% confidence that the true response rate of idarubicin in AIDS-KS is between 0% and 10%. Myelosuppression was the main observed adverse effect. Most patients, however, were heavily pretreated with chemotherapy.

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# Phase II Study of Peplomycin in Advanced Breast Carcinoma[1]

**Jungsil Ro,* Aman U. Buzdar, Laura Esparza, and G. N. Hortobagyi[2,3]**

Peplomycin is a bleomycin analog with a 3-[(*S*)-1′-phenylethylamino]-propylamine side chain, obtained by the fermentation of the actinomyces *Streptomyces verticilus* (1). In a phase I study, pulmonary toxicity was described as the major dose-limiting factor (2). From these studies, the recommended therapeutic dose for phase II trials was 5–10 mg two or three times weekly, for a total dose of 200 mg. In extensive phase II clinical studies in various solid tumors, peplomycin was found to have activity in head and neck cancer, non-small cell lung carcinoma, melanoma, prostate carcinoma, and breast cancer (3–5). The present study reports the results of a phase II study of peplomycin in advanced metastatic breast cancer.

## PATIENTS AND METHODS

Patients with histologically proven, advanced breast carcinoma who received prior chemotherapy and/or hormonal therapy and were refractory to doxorubicin-containing regimens were eligible. Written informed consent was obtained from each patient and all patients had measurable lesions, performance status $\leq 3$, life expectancy $\geq 8$ weeks, granulocyte count $\geq 2000/mm^3$, platelet count $\geq 10{,}000/mm^3$, total bilirubin $\leq 2.0$ mg/100 ml, and serum creatinine $\leq 1.5$ mg/100 ml. Patients were off all prior therapy for $\geq 3$ weeks and recovered from toxic effects from such treatments. Prestudy evaluation included history and physical examination, tumor measurement, cbc, differential and platelet count, SMA-12, x-rays, ECG, urinalysis, arterial blood gases, and pulmonary function test (FVC/DLCO). Vital capacity $\leq 2$ L or carbon monoxide diffusion capacity $\leq 60\%$ precluded participation in the study.

Peplomycin was started at a dose of 5 mg/m$^2$ iv twice weekly and escalated to 7.5 mg/m$^2$ and then to 10 mg/m$^2$ in the absence of toxic effects. An adequate trial consisted of therapy for $\geq 8$ weeks, unless severe toxic effects from drug occurred or the disease progressed rapidly during this trial period. International Union Against Cancer (UICC) criteria were followed for the evaluation of response.

## RESULTS

Patient characteristics are shown in table 1. Of 16 patients entered in the study, one was not evaluable for response because the drug was discontinued prematurely due to toxic effects. One patient had $< 50\%$ regression in tumor dimensions for 8 weeks but progressed on further treatment. The remaining 14 patients progressed on treatment. One of these 14 patients received only three doses and was taken off treatment because of rapid progression of disease. The total cumulative doses varied from 30.1 to 254.25 mg (median, 93). Repeat carbon monoxide diffusion capacity was available in seven patients; three patients had $> 10\%$ decrease in 2 months. All patients were off study due to tumor progression and free of respiratory symptoms or signs when repeat DLCO was performed. Mild to moderate gastrointestinal toxic effects occurred in 12 of 16 patients. Skin manifestations were observed in seven patients with erythematous macules or papules, blisters, and hyperpigmentation with or without pruritus. One patient was taken off the treatment due to severe skin rash and pruritus. Hematologic toxicity was negligible with this agent. Fever and chills due to drug were observed in 12 of 16 patients. No infectious complications were observed.

## DISCUSSION

Peplomycin is an analog of bleomycin, with similar antibiotic and antitumor activities but less pulmonary toxicity than bleomycin in experimental studies. Previous clinical trials reported substantial antitumor activity in various solid tumors including advanced breast

[1] Received Oct 20, 1986; revised Apr 22, 1987; accepted Apr 27, 1987.

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[3] We thank Ozella E. Walton for her assistance in the preparation of this manuscript.

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TABLE 1.—Patient characteristics and response

| Characteristic | No. of patients (%) |
|---|---|
| Total | 16 |
| Median age in yrs (range): 50 (29–67) | |
| Performance status* | |
| 0–2 | 15 (94) |
| 3 | 1 (6) |
| Previous treatment | |
| Chemotherapy (No. of doses) | |
| 2 | 1 (6) |
| 3–4 | 9 (56) |
| ≥ 5 | 6 (38) |
| Chemotherapy and radiotherapy | 14 (88) |
| Dominant sites of involvement | |
| Visceral | 12 (75) |
| Bone | 2 (12) |
| Soft tissue | 2 (12) |
| No. of metastatic sites | |
| 1 | 3 (19) |
| 2 | 5 (31) |
| ≥ 3 | 6 (38) |
| Response to therapy | |
| Stable disease | 1 (6) |
| Disease progression | 14 (88) |
| Inevaluable | 1 (6) |
| Toxic effects | |
| Nausea and vomiting | 12 (75) |
| Fever and/or chills | 12 (75) |
| Rash and/or pruritus | 7 (44) |
| Malaise or fatigue | 6 (38) |
| Anorexia | 4 (25) |
| Headache | 3 (19) |
| Diarrhea | 4 (25) |
| Mucositis | 2 (13) |
| Alopecia | 1 (6) |
| Leukopenia | 1 (6) |
| Thrombocytopenia | 1 (6) |

*UICC criteria.

cancer. Nakanishi et al (4) treated 20 patients with breast carcinoma who had had no prior chemotherapy or hormonal treatment and obtained an objective response in seven patients. Kimura et al (5) treated 12 patients with metastatic breast cancer and observed responses in two patients; both patients had had no prior chemotherapy. However, peplomycin at the dose and schedule used in the present study did not reveal significant antitumor activity in previously treated patients with advanced metastatic breast cancer. Toxic effects were tolerable.

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# 5-Fluorouracil and Cytarabine—Ineffective Treatment in Advanced Colorectal Carcinoma: A Southeastern Cancer Study Group Trial[1,2]

**Richard S. Eisenstaedt,[3,*] Barbara A. Neilan,[4] and Alfred Bartolucci[5]**

Advanced colorectal carcinoma is notoriously resistant to therapy. 5-Fluorouracil (5-FU), the most effective single agent, produces a response rate of 20% (1). As a single agent, cytarabine (ARA-C) has not shown much activity in humans with colon cancer when administered by bolus injection (2,3) and Gailani et al (4) reported simultaneous 5-FU plus ARA-C to be no more effective than 5-FU alone. However, prolonged infusion may augment the antineoplastic effect of a cell cycle phase-specific drug like ARA-C without dramatically enhancing toxicity, and the alteration in nucleotide pool sizes might then potentiate activity of subsequently administered 5-FU. ARA-C, in inhibiting the conversion of cytidine to deoxycytidine, reduces levels of deoxynucleotides. The resultant decrease in dUMP increases thymidylate synthetase inhibition by FdUMP, adding to the expected inhibition of this enzymatic step by 5-FU alone (5). The purpose of this study is to evaluate response to a 5-day infusion of ARA-C followed 1 day later by iv 5-FU in patients with metastatic colon cancer.

## METHODS

Eligibility criteria for this study included: (*a*) histologically diagnosed colorectal carcinoma with suspected metastases; (*b*) measurable lesion on physical examination, chest x-ray, or other imaging device; (*c*) patients may have received prior irradiation but not within the last month, and may not have received prior chemotherapy; and (*d*) granulocyte count $> 2000/mm^3$, platelet count $> 100,000/mm^3$, and Karnofsky performance score $> 60\%$.

A treatment cycle consisted of ARA-C at 37.5 $mg/m^2$ iv by continuous infusion for 24 hours on Days 1–5; and 5-FU at 250 $mg/m^2$ on Day 7. Each cycle was repeated at 29-day intervals.

## RESULTS

Twenty-five patients were entered in the study (table 1). Five patients were not evaluable, two because of inadequate reported data, two because of inadequate objective tumor measurements, and one because of early death. Of the remaining 20 patients, one had partial response, nine had no progression, and ten had disease progression during treatment. Thus, the overall response rate was 5% for evaluable patients.

Toxicity was significant with the regimen employed. Twenty-nine percent of the patients had granulocyte

TABLE 1.—Patient characteristics

| Characteristic | No. of patients |
|---|---|
| Total | 25 |
| Ineligible | 2 |
| Inadequate follow-up data | 2 |
| Early death | 1 |
| Evaluable | 20 |
| Median age in yrs: 61 | |
| Response | |
| Partial | 1 |
| Stable disease | 9 |
| Disease progression | 10 |
| Hematologic toxicity ($cells/mm^3$) | |
| Granulocyte count | |
| 1000–1500 | 4 |
| 500–1000 | 9 |
| < 500 | 1 |
| Platelet count | |
| 100,000–150,000 | 2 |
| 50,000–100,000 | 3 |
| 25,000–50,000 | 1 |
| < 25,000 | 1 |

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counts < 1500/mm$^3$, 43% had granulocyte counts < 1000/mm$^3$, and 5% had granulocyte counts < 500/mm$^3$. Thrombocytopenia was noted in 25% of the patients, with 5% having severe platelet count depression < 25,000/mm$^3$. Mild nausea and vomiting were noted in almost half of the patients treated. There were no treatment-related deaths.

## DISCUSSION

The combination of ARA-C plus 5-FU was ineffective in this patient population with metastatic colon cancer who had never received prior chemotherapy. The low response rate, 5%, in conjunction with significant marrow toxicity suggests that dose modifications are unlikely to augment response. New drugs should be evaluated for efficacy in this disease.

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# Phase II Study of Carmustine in Advanced Upper Aerodigestive Cancer[1]

**Edward T. Creagan,* John H. Edmonson, Ronald L. Richardson, and Daniel J. Schaid[2]**

Cisplatin and cisplatin-based polychemotherapy produce regression rates of approximately 30% and 70%, respectively, in advanced upper aerodigestive cancer (1). Responses are usually transient, have not meaningfully influenced survival, and may be accompanied by substantial clinical sequelae (1,2). Durable palliation is most evident for patients demonstrating a complete response. We report here a phase II trial of carmustine in patients with biopsy-proven advanced upper aerodigestive cancer (table 1) in view of data indicating some activity against these neoplasms (3,4). Each patient had evaluable disease; no prior nitrosourea exposure; and adequate hematologic (wbc count $> 4100/mm^3$; platelet count $> 130,000/mm^3$), renal (creatine $< 1.5$ mg/dl), and hepatic (normal direct bilirubin) parameters. The iv dose of carmustine was 200 mg/m$^2$ administered over 2–3 hours with 5% dextrose in water at 6-week intervals.

One of 18 patients evaluated had objective regression of evaluable disease using conventional criteria (2). This patient had squamous cell carcinoma. One patient has not yet returned for follow-up. Median time to disease progression was 1.5 months, and median survival was 4 months.

Toxic effects were primarily hematologic and gastrointestinal. Among 14 patients with interim hematologic data, six (43%) had a wbc count nadir $< 4000/mm^3$ and ten had a platelet count nadir $< 130,000/mm^3$. The median wbc count nadir was $4.3 \times 10^3/mm^3$, and one patient had a wbc count $< 2000/mm^3$. Five patients had moderate to severe anorexia, and moderate to severe nausea and vomiting affected three and two patients, respectively. We did not detect any apparent hepatorenal toxic effects.

We could not demonstrate any therapeutic value from carmustine utilizing this dose and schedule among extensively pretreated patients with advanced upper aerodigestive cancer of diverse histologies. This trial cannot address the issue if more intense myelosuppression would have enhanced efficacy, nor can we assess the impact of carmustine among patients with less extensive prior therapy. Nevertheless, the occurrence of only one objective regression concomitant with fulminant disease progression would obviate further evaluations of the current carmustine regimen in this patient population.

TABLE 1.—Patient and tumor characteristics

| Characteristic | No. of patients |
|---|---|
| Sex | |
| Male | 11 |
| Female | 8 |
| Median age in yrs (range): 57 (35–70) | |
| Performance status* | |
| 0, 1 | 9 |
| 2, 3 | 10 |
| Carcinoma | |
| Squamous cell | 16 |
| Nonsquamous cell† | 3 |
| Prior treatment | |
| Chemotherapy | 13‡ |
| Radiation therapy | 19 |

*Eastern Cooperative Oncology Group criteria.

†Nonkeratinizing squamous cell carcinoma (lymphoepithelioma) (2 patients); and mucoepidermoid and adenoid cystic carcinoma (1 patient each).

‡Platinum-based polychemotherapy (10 patients). No. of prior regimens: 1 (7 patients); 2 (3 patients); 3 (1 patient); and 4 (2 patients).

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# Phase II Trial of Esorubicin in Patients With Advanced Non-Small Cell Lung Cancer[1,2,3]

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Esorubicin is an anthracycline analog which differs from its parent compound by the absence of a hydroxyl group at the 4′ position of the amino sugar. The drug has demonstrated preclinical antitumor activity (1,2) and no significant cardiotoxicity in model systems (2). In a phase I trial among adult patients with advanced solid tumors who received a bolus dose every 3 weeks, leukopenia, thrombocytopenia, nausea, vomiting, alopecia, and local urticarial reactions were observed (3). Based on this information, a phase II trial of esorubicin was undertaken in patients with advanced non-small cell lung cancer.

## PATIENTS AND METHODS

Thirty-eight patients with stage III or IV non-small cell lung cancer were entered in this study. Eligibility criteria included a pathologic specimen review; the presence of nonirradiated measurable or evaluable indicator lesions; Karnofsky performance status (KPS) $\geq 50\%$; normal hepatic, renal, and cardiac functions; leukocyte count $\geq 4000/\mu l$; and platelet count $\geq 120{,}000/\mu l$. Esorubicin was administered iv every 21 days at a starting dose of 30 mg/m$^2$ for previously untreated patients with a KPS $> 70\%$; all others were started at 25 mg/m$^2$. The dose in subsequent courses was increased by 5 mg/m$^2$ if the maximum toxic effect was 0 or 1+ in the prior course and decreased by 5 mg/m$^2$ if 3+ or 4+ toxic effects were observed. Standard response and toxicity criteria were used (4).

## RESULTS

Patient characteristics are shown in table 1. Thirty-six of the 38 patients (95%) received an adequate trial. One inadequately treated patient had no change in his indicator lesion and no toxic effects during a single course. The other patient did not have a follow-up examination. None of the previously treated patients had received anthracycline compounds. A median of two courses (range, one to 15) of esorubicin were given. One previously untreated man (initial KPS of 90%) with a nonmeasurable indicator lesion had a major objective response (4) lasting 8.8 months. All other patients had no change or disease progression.

Leukopenia, thrombocytopenia, nausea, vomiting, alopecia, and stomatitis were the most common toxic effects seen. The percentage of patients with the maximum toxicity grades for each of these effects is shown in table 2. Both leukocyte and platelet count nadirs were noted on Days 10–14 after therapy. Diarrhea (one patient) and urticarial local reactions (four patients) were also seen. Three patients were hospitalized for fever during a period of drug-induced leukopenia; one died. No patient developed clinical congestive heart failure. Serial radionuclide left ventriculograms obtained in nine patients who received 70-488 mg/m$^2$ of esorubicin revealed no significant diminution in resting ejection fractions.

## DISCUSSION

With only one of 36 adequately treated patients having had a major response, the predicted true response rate for esorubicin in non-small cell lung cancer patients

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TABLE 1.—Patient characteristics and therapeutic results

| | Total patients | Prior chemotherapy | No prior chemotherapy |
|---|---|---|---|
| No. of patients | | | |
| Entered | 38 | 20 | 18 |
| Adequate | 36 | 19 | 17 |
| Median age in yrs | 60 | 59 | 60 |
| Sex (%) | | | |
| Male | 67 | 63 | 71 |
| Female | 33 | 37 | 29 |
| KPS (%) | | | |
| 80-90 | 50 | 47 | 53 |
| 50-70 | 50 | 53 | 47 |
| Histologic subtype (%) | | | |
| Adenocarcinoma | 78 | 84 | 71 |
| Epidermoid carcinoma | 14 | 11 | 18 |
| Large cell carcinoma | 8 | 5 | 11 |
| Stage IIIA or IIIB/IV (%) | 17/83 | 21/79 | 12/88 |
| Bone metastases (%) | 25 | 37 | 12 |
| Prior radiotherapy, any site (%) | 50 | 42 | 59 |
| Major response rate (%) | 3 | 0 | 6 |
| 95% confidence limits for predicted rate (%) | 0.1-16 | 0-15 | 0.3-25 |

TABLE 2.—Toxic effects

| Toxic effect | World Health Organization scale* | | | | |
|---|---|---|---|---|---|
| | 0 | 1+ | 2+ | 3+ | 4+ |
| Leukopenia | 11 | 20 | 22 | 36 | 11 |
| Thrombocytopenia | 59 | 19 | 14 | 8 | 0 |
| Nausea and vomiting | 78 | 14 | 8 | 0 | 0 |
| Alopecia | 86 | 8 | 6 | 0 | 0 |
| Stomatitis | 88 | 6 | 3 | 3 | 0 |

*Values = % of patients with maximum toxicity grade as indicated (ref 4). Total No. of Patients = 36.

is $\leq 16\%$ ($P = 0.05$). For patients who had not received prior chemotherapy, the observed major response rate was 6% (95% confidence limits, 0.3%-25%). These results are consistent with those of Kaplan et al (5), who reported no major responses in a similar group of 38 non-small cell lung cancer patients. Esorubicin, in the dose and schedule used, had only marginal activity in this group of patients with non-small cell lung cancer.

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## Transient Tumor Marker Surge Following Chemotherapy of Testis Tumors

The Brief Report by Horwich and Peckham (1) in the November 1986 issue of *Cancer Treatment Reports* was interesting but deserves further comment. Gips et al (2) have discussed the problem of marker "surge" following initiation of chemotherapy. They explain the surge as the effect of damage to a large number of tumor cells in mitosis resulting in the release of stored human chorionic gonadotropin (HCG). The best method of documenting this phenomenon is to measure tumor markers before chemotherapy and at 2-day intervals following chemotherapy. A rise of HCG during the first 2–6 days to 177.3%–407.5% of the initial level and a descent to 17.9%–33.7% of the initial level at Day 10 of therapy are the established marker patterns of the release of stored HCG in tumor cells. In the case of established tumor resistance, there is an autonomous unremitting increase of HCG activity.

I believe that Horwich and Peckham (1) performed beta human chorionic gonadotropin (BHCG) levels at weekly intervals, which is too infrequent and may miss completely the marker surge occurring during the first week. Also, this surge is due to tumor lysis and does not have adverse prognostic implications. They mention that one of the nine patients who had a marker surge did relapse. It is possible that this patient was never in complete remission. One method of establishing complete remission in these patients would be to make sure there is no marker surge after chemotherapy, especially when BHCG levels are drawn either daily or on alternate days for the first week to 10 days after chemotherapy. Doing markers at weekly intervals would be inadequate to document the marker "surge," thereby allowing persisting tumor cells to go undiagnosed.

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### *Reply*

The letter by M. R. Shetty quotes a single case report of a patient with gestational choriocarcinoma treated with pulses of methotrexate as a single agent and then with a combination of vinblastine, methotrexate, and dactinomycin. It is wrong to assume that the same kinetics of marker surge will apply to the use of all chemotherapy combinations or to the treatment of germ cell tumors of the testis. We have analyzed daily serum marker levels in five patients treated with bleomycin, etoposide, and cisplatin (1). In three patients no surge was seen, even on the initial treatment course, and in two other patients the peak occurred on Day 5 of this 5-day treatment schedule. Additionally, in advanced or recurrent germ cell tumors, we have used an intensive 4-week induction chemotherapy schedule containing weekly bleomycin, vincristine, and cisplatin and the HCG (human chorionic gonadotropin) surge peak on this schedule is commonly on Day 14. It seems probable that the timing of the surge is drug-schedule dependent. The cause of the surge in human tumors has not been established and may well include marker release following tumor cell lysis or, as discussed in our article, differentiation of tumor cells. The value of this phenomenon in diagnosing persisting tumor cells following later courses of chemotherapy has not been evaluated.

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## Pulmonary Toxicity of Lomustine[1]

Interstitial pneumonitis and fibrosis are frequent serious side effects of carmustine, and have also been reported with two other nitrosoureas, semustine and chlorozotocin (1). To date, few cases of lomustine (CCNU)-associated pulmonary toxicity have been published. In several of these, the patients had received chest irradiation, or other chemotherapeutic agents known to cause pulmonary fibrosis. The following case reinforces the premise that CCNU alone may cause fatal pulmonary toxicity.

An 80-year-old man with no prior history of pulmonary disease underwent a right hemicolectomy in May 1982 for Duke's C2 adenocarcinoma of the cecum. Asymptomatic bilateral lower lobe pulmonary nodules were noted on chest x-ray in November 1982, and the patient was followed without treatment for 27 months. Sequential chest radiographs revealed slowly progressive enlargement of the nodules, and in late 1984 a biopsy-proven left cheek metastasis developed. Subsequently, the patient received single-agent 5-fluorouracil chemotherapy (February to August 1985) without incident. This therapy was stopped because of continued enlargement of the pulmonary nodules. The patient then agreed to participate in an institutional phase II study of weekly low-dose CCNU. He received CCNU, 60–80 mg orally, every week for 15 weeks, for a total dose of 1020 mg (443 mg/m$^2$). Treatment was discontinued in December 1985 because of thrombocytopenia and enlargement of the left cheek metastasis. During the entire period of chemotherapy, the patient's chronic medications of nifedipine, nitrobid, hydrochlorothiazide, and cimetidine were continued. No new medications were added to this regimen. The patient was admitted February 11, 1986, for evaluation of 1 week of low-grade fever, cough, dyspnea, and the new onset of bilateral patchy pulmonary infiltrates. Arterial blood gases on room air were: $PO_2$ 58, $PCO_2$ 35, and pH 7.51. Erythromycin and trimethoprim/sulfamethoxazole therapy was started. Serial chest radiographs showed worsening of the pulmonary infiltrates and the patient experienced increasing respiratory insufficiency with severe hypoxemia, even on a 100% oxygen nonrebreather mask. The patient was not intubated in accordance with his wishes and died of respiratory failure on February 16, 1986.

Autopsy findings were remarkable for metastatic adenocarcinoma involving the lower lobes of both lungs, the liver, the left kidney, and the left adrenal gland. The remainder of the pulmonary parenchyma was firm and red-brown with areas of pale consolidation. Microscopically, these areas showed a range of alveolar lining cell hypertrophy and hyperplasia, hyaline membrane formation, focal chronic inflammation, and areas of early diffuse fibrosis. No acute inflammatory infiltrates and no viral inclusions were seen.

The above findings are characteristic of the histopathologic syndrome termed diffuse alveolar damage. The patient had no evidence of any of the non-drug-related causes of this entity which include infection, shock, inhalant injury, and irradiation (1). Neither 5-fluorouracil nor any of his chronic medications are associated with this problem; therefore, we believe that CCNU is the responsible agent.

Weiss et al (2) reviewed the subject of nitrosourea-induced pulmonary toxicity in 1981, proposing that the absence of reported CCNU toxicity to that date was referable to the fact that few patients received a large enough total dose to acquire the problem. Since then, several reports of CCNU toxicity have been published. Cordonnier et al (3) reported two pathologically confirmed and three presumed cases of CCNU pulmonary fibrosis, including four fatalities. The pathologically confirmed cases had received 2000 and 2120 mg of CCNU, respectively, and each of the other cases had received at least 1100 mg/m$^2$. Four of the patients had also received either cytoxan or busulfan, known pulmonary toxins. Vats et al (4) reported a 7½-year-old male who received CCNU, 1170 mg/m$^2$, for treatment of a glioma. He received no other pulmonary toxins and died of pulmonary fibrosis 20 months after the drug was discontinued. Dent (5) reported a 52-year-old male who had previously received 20 Gy of spinal irradiation for a glioma. At recurrence he received a total of 600 mg of CCNU. Shortly after the last dose, the patient developed respiratory failure and died. Autopsy revealed pulmonary changes compatible with drug toxicity.

In a recent review of pulmonary toxicity associated with combined-modality therapy for small cell lung cancer (6), 28% of 40 patients receiving concurrent radiation therapy and chemotherapy developed life-threatening pulmonary toxicity. These patients received 250 mg/m$^2$ of CCNU, as well as cyclophosphamide and methotrexate. The exact role of CCNU in this setting is unclear, but it may have a synergistic effect with the other pulmonary toxins.

We conclude that CCNU therapy is associated with a nonnegligible risk of pulmonary toxicity. Risk factors appear to include a large cumulative dose, and the concurrent administration of other pulmonary toxins. Our case confirms, however, that CCNU alone, even at a modest total dose, can result in fatal pulmonary toxicity.

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[1] The opinions or assertions herein are the private views of the authors and are not to be construed as official or as reflecting the view of the Department of the Army or the Department of Defense.


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## Delirium and Severe Sedation in Patients With Terminal Cancer

Delirium and dementia are recognized complications of cancer (1). However, different papers have reported an incidence between 8% (2) and 85% (3). In addition to delirium, terminal cancer patients can also present severe sedation. These symptoms make management of the patients very difficult, and knowledge about their incidence and natural history may have implications in the planning of care and clinical research in terminal care. We recently reviewed the cases of 30 consecutive patients who died after being admitted to the hospital for at least 1 week under our care. All the patients had metastatic cancer. Mean age was 54 ± 6 years and 14 patients were female. None of the patients was known to have brain metastases and all were receiving narcotics for the treatment of cancer pain. All patients were assessed daily by at least one of the authors. The diagnosis of delirium was made in the presence of a confusional state with or without hallucinations or hyperactivity. Severe sedation was diagnosed when it was impossible to establish a dialogue with the patient. Cases of delirium or severe sedation that occurred during the last 48 hours of life were excluded. The results of laboratory tests and dose and type of medication were collected from the charts. The degree of pain control during the admission was considered as adequate (no pain most of the time, less than two extra doses of analgesic per day), or inadequate (pain present most of the time or requirement for two more doses of extra analgesic per day). A total of 23 patients (77%) developed impaired mental status (delirium in 16 and severe sedation in seven) > 48 hours before death.

Symptoms were detected an average of 9 ± 6 days before death. Blood tests (cbc, SMA-12, and electrolytes) were performed in all patients with impaired mental status. A computed tomography of the brain (nine patients) or nuclear brain scan (one patient) was performed in ten of 23 patients (43%) and results were normal in all cases. One more patient had a central nervous system autopsy that was found to be normal.

The cause of impaired mental status was found in five patients (21%). These patients are summarized in table 1.

In the remaining 18 cases, patients had no abnormalities in blood tests that could explain the mental changes, and the discontinuation of all drugs, except the analgesics, did not result in any improvement in the symptoms. Eight of these patients (44%) had a computed tomography of the brain that was normal. Pain control was considered adequate in 21 of 23 patients with impaired mental status (91%), versus three of seven patients without mental impairment (43%, $P < 0.05$).

In nine of 23 patients with mental impairment, the dose of narcotics was decreased before death (39%): in one case there was significant improvement in delirium (previously described); in three cases the dose had to be reincreased because of pain with no improvement in

TABLE 1.—Characteristics of patients in whom the cause of impaired mental state was found

| Patient No. | Clinical presentation | Cause | Treatment | Response |
|---|---|---|---|---|
| 1 | Severe sedation | Hyponatremia (20 mEq/L) | Water restriction | Improvement |
| 2 | Severe sedation | Hypercalcemia (13.5 mEq/L) | Hydration | Nil |
| 3 | Severe sedation | Diazepam (20 mg/day) | Discontinuation | Improvement |
| 4 | Delirium | Amytriptyline (150 mg/day) | Discontinuation | Improvement |
| 5 | Delirium | Morphine (350 mg/day) | Change to hydromorphone | Improvement |

mental status; and in five cases the patients continued on the reduced dose of narcotic until death with no improvement. In patients without mental impairment, the dose of narcotics was decreased in none of the seven patients ($P < 0.05$).

In this retrospective study of 30 patients with terminal cancer who died in our hospital, we found that 23 (77%) developed serious mental impairment an average of > 1 week before death. The cause of mental impairment could only be found in five cases (21%), and significant improvement was found in four of these cases.

Though delirium and dementia are recognized complications of cancer, their incidence in cancer patients has been reported in 8%–85% (2,3). One series of organic brain syndrome accounted for 40% of the referrals of cancer patients to a psychiatrist (4). This difference in findings can probably be ascribed to difference in populations, method of psychological assessment, and intensity of follow-up. Early dementia can be misdiagnosed, usually as depression (3–6), and patients can be treated with antidepressants, which can worsen the symptoms (6). Because of the retrospective nature of our study, it is possible that the incidence of organic brain syndrome in our population was even higher than the one we report. It seems obvious that during the last hours of life almost all patients develop organic brain syndrome that results from the generalized systems failure (massive renal or liver failure, sepsis, hypoxemia, low brain profusion, etc). In these patients, the cause of the symptoms is easily identifiable because of the catastrophic underlying mechanism and the fact that the life span can accurately be estimated in hours or days. For this reason we decided not to include in our study those patients who develop organic brain syndrome within 48 hours before death. We were rather interested in the presence of organic brain syndrome or severe sedation in patients who had terminal cancer but were not obviously in the last hours of life. Our findings suggest that the communication between the patient and the family and medical staff during the last week of life is usually almost impossible, and such things as informed consent for therapeutic or research procedures, or resuscitation, may be impossible to obtain reliably at that stage. The final emotional stage suggested in many dying patients (3) may perhaps represent the development of an organic brain syndrome with its cognitive consequences. Our results suggest that the reason for confusion or sedation frequently remains unknown. Although narcotics are probably responsible for at least some of our cases of confusion or sedation, the dose usually cannot be decreased without worsening the pain syndrome. Our results suggest that a prospective trial using a sensitive method for the diagnosis of early stages of delirium is highly justified. If our results are confirmed, they will probably have impact on the planning of treatment and research in this population.

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## Acute Thyroiditis as a Complication of Chemotherapy Administration Through a Hickman Catheter

The utilization of indwelling central venous catheters such as the Hickman catheter is becoming more widespread, especially in the care of oncology patients. Such catheters have many advantages, but a number of complications have been observed. We describe a complication not previously reported: acute inflammation of the thyroid secondary to infusion of chemotherapeutic agents through the inferior thyroid vein, associated with thrombus about the Hickman catheter tip preventing outflow into the superior vena cava.

### CASE REPORT

A 54-year-old white female with newly diagnosed acute myelogenous leukemia was admitted in October 1986 for induction chemotherapy with daunorubicin, cytarabine, and 6-thioguanine. The patient received the daunorubicin and cytarabine via a peripheral iv line, but because of subsequent difficulties with venous access, a double-lumen Hickman catheter was placed via the right external jugular vein. Intraoperative chest x-ray revealed catheter tip location at the right brachio-

cephalic vein-superior vena cava junction. The catheter tip location could not be improved despite multiple attempts at repositioning. As there was good antegrade and retrograde flow via both limbs of the catheter, the procedure was terminated. Subsequently, iv fluids were infused easily through the catheter but blood return was episodic. It was felt that the catheter tip was intermittently against a vessel wall.

When follow-up bone marrow examination demonstrated failure to achieve a remission, the patient was begun on a second induction course with the same drugs. Infusional cytarabine (at approximately 12 mg per hour) was started via the Hickman catheter and the first day's dose of daunorubicin (74 mg) was administered through the catheter that evening without event. However, on the following morning the patient noted a sensation of fullness across her lower neck. Physical examination at that time did not demonstrate any appreciable swelling and only minimal tenderness over the lower neck. Because of concern that the catheter may have migrated up an internal jugular vein, a chest x-ray was obtained but the catheter tip location was unchanged. Cervical films failed to demonstrate soft tissue swelling. Thyroid function tests were normal. The patient had no prior history of thyroiditis or other thyroid problems. That evening, after approximately one-third (24 mg) of the second daunorubicin dose had been administered, the patient began complaining of a burning sensation in her neck. The daunorubicin was immediately discontinued and another chest x-ray was obtained, with the catheter tip location again unchanged. Nevertheless, all infusions through the Hickman catheter were stopped.

The patient's lower neck progressively swelled through the night and it became clear that this represented diffuse enlargement of her thyroid. By the next morning her goiter was quite large (approximately 50–60 g), firm, warm, and exquisitely tender to palpation. Her maximal temperature during this time was 38°C orally, and her other vital signs were stable.

A venogram through the Hickman catheter was obtained later that day. The study demonstrated the catheter tip to be in the right brachiocephalic vein near its junction with the superior vena cava. There was extensive clot about the tip and no flow into the superior vena cava. Instead, there was runoff of dye superiorly through the inferior thyroid vein.

By the following day her thyroid was much less tender and had already shrunken considerably. The gland continued to decrease in size over several days until it was no longer palpable, and her cervical tenderness resolved several days after that. Serial thyroid function tests remained stable and the patient showed no clinical evidence of either hypo- or hyperthyroidism. Repeated serum calcium and phosphorus determinations showed no evidence of parathyroid dysfunction.

The patient was returned to the operating room and the cervical incision was reopened. The catheter was withdrawn from the external jugular vein, which was then ligated. The cervical incision was extended medially and the right internal jugular vein exposed. The catheter was then inserted via a purse-string venotomy. Chest x-ray revealed catheter tip at the superior cava-right atrial junction. In this position the catheter functioned well for both infusions and blood drawing. The patient completed the remainder of her chemotherapy uneventfully.

## DISCUSSION

In 1973 Broviac et al (1) described the placement of a silicone rubber catheter into the superior vena cava to provide long-term venous access for patients requiring parenteral nutrition over weeks or months. In 1979 Hickman et al (2) reported the use of a larger-bore catheter which could also be used for transfusion of blood products and for withdrawing blood. Multiple variants of these devices are now available and provide convenient and generally reliable venous access for the administration of chemotherapeutic agents, blood products, parenteral nutrition, antibiotics, and other iv fluids. They also usually allow venous blood sampling. The use of such catheters continues to increase and as it does new complications may be seen. We report the complication of acute chemical thyroiditis secondary to selective perfusion of the thyroid with chemotherapy via the inferior thyroid vein.

Thrombosis is not an uncommon catheter-related problem, and, together with infection, represents the most common major complication (3). One clue to the presence of clot is the so-called "one-way catheter" in which the catheter is readily flushed, but it is difficult to withdraw blood (4). In our case, difficulty in blood drawing was present from the time of catheter insertion, leading us to suspect positioning of the tip against a vessel wall rather than thrombus.

Ordinarily, Hickman catheters are placed via the cephalic, jugular, or subclavian veins under local anesthesia. The cephalic approach is often tedious and may involve significant patient discomfort. The percutaneous subclavian approach risks complications including pneumothorax and hemothorax. In our patient, the jugular approach was selected in part because of thrombocytopenia. The right external jugular vein affords a more direct route into the central venous circulation than the left. This vessel is easily exposed just above the clavicle with minimal dissection and with minimal risk of bleeding.

Hickman catheter tip displacement is not rare. One study described an incidence of catheter displacement into the internal jugular vein approaching 7% (5). Another report describes a catheter initially in good position, subsequently looped up inside the superior vena cava and wedged into the axillary vein (6). In our patient, migration of the catheter was considered when the patient began complaining of neck discomfort, but this was not borne out on chest x-ray examination. Venography through the catheter disclosed the true nature of the problem.

This case report presents a previously unreported complication to be considered when a patient receiving chemotherapy through a Hickman catheter complains of a new onset of neck discomfort or swelling in the area of the thyroid gland. While chest x-ray may confirm the position of the catheter tip, venography through the catheter is more precise and should be performed promptly to confirm the presence of thrombus about the catheter tip. The placement of the catheter tip well within the superior vena cava should be the operative goal and will reduce the incidence of complications. When utilizing the external jugular approach, if suboptimal catheter tip location cannot be improved by catheter manipulation, the external jugular approach should be abandoned and the ipsilateral internal jugular vein cannulated.

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March 27, 1987



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## "Cisplatin Plus Etoposide as Second-Line Treatment in Advanced Ovarian Carcinoma"

We would like to comment on the letter of M. S. Piver (1) concerning our paper (2). In our paper we emphasize that cisplatin-etoposide is an effective regimen in very heavily pretreated patients, although they were not necessarily pretreated with cisplatin. In fact, all patients in our series were refractory to a doxorubicin-cyclophosphamide regimen and therefore the 40% complete plus partial response must be considered more than satisfactory. Moreover, our series cannot be compared to that of Wiltshaw and Kroner (3) because of the different pretreatment characteristics. In the latter series, no patient had received a doxorubicin-containing regimen as first-line chemotherapy but only alkylating agents with or without radiation therapy.

In conclusion, we agree with Piver that cisplatin regimens are more active in first-line chemotherapy in ovarian cancer as demonstrated by Omura et al (4), but we underline the fact that cisplatin remains the only active agent for second-line chemotherapy. Therefore, it is more than justified to administer cisplatin plus etoposide based on their well-known synergism. The very interesting results recently reported by Barlow and Lele (5) also support this conclusion. Naturally, a randomized study is needed to demonstrate the superiority of cisplatin-etoposide as compared to cisplatin alone.

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1. PIVER MS. "Cisplatin plus etoposide as second-line treatment in advanced ovarian carcinoma." Cancer Treat Rep 70:1466, 1986.
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# ANNOUNCEMENTS

### Continuing Education Program: Upper Gastrointestinal Cancer

***Cleveland, Ohio, September 9, 1987***

*For further information, contact:* Lisa Politi, Continuing Education Program Coordinator, Department of Continuing Education, The Cleveland Clinic Educational Foundation, 9500 Euclid Ave, Rm TT3-301, Cleveland, OH 44106 [telephone: 444-5696 (local); 800-762-8172 (Ohio); and 800-762-8173 (outside Ohio)].

### First International Paediatric Oncology Nursing Meeting

***Jerusalem, Israel, September 13–18, 1987***

*For further information, contact:* Prof. Rina Zaizov, Chairperson, PO Box 983, Jerusalem 91009, Israel.

### Clinical Symposium in Cancer Oncology

***Los Angeles, California, September 17–18, 1987***

*For further information, contact:* Beverly Johnson, Program Specialist, University of Southern California School of Medicine, Postgraduate Division, 1975 Zonal Ave, KAM 314, Los Angeles, CA 90033 (telephone: 213-224-7051).

### Midwest Regional Oncology Conference III

***Kansas City, Missouri, September 24–26, 1987***

*For further information, contact:* Susie Fisher, Conference Planner, Center for Continuing Education, St. Luke's Hospital of Kansas City, Wornall Rd at 44th, Kansas City, MO 64111 (telephone: 816-932-2301).

### Second International IST Symposium: Multimodality Treatment of Ovarian Carcinoma

***Genoa, Italy, September 24–26, 1987***

*For further information, contact:* P. F. Conte, MD, Scientific Secretariat, Istituto Nazionale per la Ricerca sul Cancro, V. le Benedetto XV-10, 16132 Genova, Italy (telephone: 010-352828/352803).

### Hodgkin's Lymphoma—Recent Advances in Basic and Clinical Research International Symposium

***Cologne, Federal Republic of Germany, October 2–3, 1987***

*For further information, contact:* Prof. Dr. V. Diehl, Studiensekretariat Morbus Hodgkin, Medizinische Universitätsklinik, Joseph Stelzmann Str. 9, D-5000 Cologne 41, Federal Republic of Germany.

### Continuing Education Program: The Challenges of Oncology Nursing

***Cleveland, Ohio, October 7–9, 1987***

*For further information, contact:* Lisa Politi, Continuing Education Program Coordinator, Department of Continuing Education, The Cleveland Clinic Educational Foundation, 9500 Euclid Ave, Rm TT3-301, Cleveland, OH 44106 [telephone: 444-5696 (local); 800-762-8172 (Ohio); and 800-762-8173 (outside Ohio)].

### Current Concepts in Medical Oncology 1987

***New York, New York, October 19–23, 1987***

*For further information, contact:* Roberto Fuenmayor, CME Conference Planner, Memorial Sloan-Kettering Cancer Center, 1275 York Ave, New York, NY 10021 (telephone: 212-794-6754).

### Principles of Epidemiology

***Washington, DC, October 28–30, 1987***

*For further information, contact:* The New England Epidemiology Institute, Department SC-4, PO Box 57, Chestnut Hill, MA 02167 (telephone: 617-734-9100).

### Second Conference on Immunity to Cancer

***Williamsburg, Virginia, November 9–11, 1987***

*For further information, contact:* Carole M. Kirby, Conference Coordinator, Biological Response Modifiers Program, Division of Cancer Treatment, National Cancer Institute-Frederick Cancer Research Facility, Bldg 567, Rm 138, Frederick, MD 21701 (telephone: 301-698-1418).

### Cincinnati Cancer Conference VI: Sarcoma

***Cincinnati, Ohio, November 13–14, 1987***

*For further information, contact:* Thomas J. O'Connor, Continuing Medical Education, Bethesda Hospital, Inc, 619 Oak St, Cincinnati, OH 45206 (telephone: 513-569-6339).

### Cincinnati Cancer Conference VI: Nursing Issues—Sarcoma: Survival and Quality of Life

***Cincinnati, Ohio, November 13–14, 1987***

*For further information, contact:* Bethesda Hospital, Inc, Nursing Staff Development, 619 Oak St, Cincinnati, OH 45206 (telephone: 513-569-6339).

### First International Breast Cancer Chemoprevention Workshop-Conference

***New York, New York, November 20, 1987***

*For further information, contact:* Jaclyn Silverman, Chemoprevention Workshop Coordinator, Division of Medical Oncology, Mount Sinai Medical Center, One Gustave L. Levy Place, New York, NY 10029 (telephone: 212-369-5440 or 213-650-6772).

### Second International Congress on Neo-Adjuvant Chemotherapy

***Paris, France, February 19–21, 1988***

*For further information, contact:* Service d'Oncologie Médicale, Professeur Claude Jacquillat, 47, boulevard de l'Hôpital, 75651 Paris Cedex 13, France.

### Arizona Cancer Center 6th Winter Symposium: Advances in Hematologic Malignancies and Bone Marrow Transplantation

***Snowbird, Utah, March 5–12, 1988***

*For further information, contact:* Mary Humphrey, Conference Coordinator, Arizona Cancer Center, Tucson, AZ 85724 (telephone: 602-626-2276).

### Fifth World Conference on Lung Cancer

***Interlaken, Switzerland, August 28–September 1, 1988***

*For further information, contact:* Rudolf A. Joss, MD, Secretary General, Institute for Medical Oncology, Inselspital, CH-3010 Bern, Switzerland.

### Yearly Meeting of the German Institute for Laser Medicine

***Vienna, Austria, October 5–8, 1988***

*For further information, contact:* Doz. Dr. A. Tuchmann, c/o Wiener Medizinische Akademie, Alser Strasse 4, A-1090 Vienna, Austria (telephone: 0222-427165).

### 13th Congress of the European Society for Medical Oncology

***Lugano, Switzerland, October 30–November 1, 1988***

*For further information, contact:* F. Cavalli, MD, Division of Oncology, Ospedale San Giovanni, 6500 Bellinzona, Switzerland.

### Workshop on Progress in Regional Cancer Therapy

***Vienna, Austria, November 3–5, 1988***

*For further information, contact:* Doz. Dr. R. Jakesz, Doz. Dr. H. Rainer, c/o B. Schick, Abteilung für Experimentelle Chirurgie der I. Chirurgischen Universitätsklinik, Alser Strasse 4, A-1090 Vienna, Austria (telephone: 0222-4800/2301).

# Cancer Treatment Reports

## ORIGINAL ARTICLES


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*681* Concurrent Cisplatin and Etoposide With Radiotherapy in Locally Advanced Non-Small Cell Lung Cancer

*685* Response to Tamoxifen and Fluoxymesterone in a Group of Breast Cancer Patients With Disease Recurrence After Cessation of Adjuvant Tamoxifen

*689* High-Dose Iv Thiotepa and Cryopreserved Autologous Bone Marrow Transplantation for Therapy of Refractory Cancer

*697* In Vitro Chemosensitivity of Human Lung Cancer Cell Lines

*705* Multivesicular Liposomes Containing Cytarabine Entrapped in the Presence of Hydrochloric Acid for Intracavitary Chemotherapy

*713* Phase II Evaluation of Teniposide and Ifosfamide in Refractory Adult Acute Lymphocytic Leukemia: A Southwest Oncology Group Study

*717* Scheduling of Vincristine: Drug Accumulation and Response of Xenografts of Childhood Rhabdomyosarcoma Determined by Frequency of Administration

*723* Platinum Analogs in Recurrent and Advanced Head and Neck Cancer: A Southwest Oncology Group and Wayne State University Study

*727* Combination Chemotherapy With a New Folate Analog: Activity of 10-Ethyl-10-deaza-aminopterin Compared to Methotrexate With 5-Fluorouracil and Alkylating Agents Against Advanced Metastatic Disease in Murine Tumor Models

*733* Cardiotoxicity of 5-Fluorouracil


## REVIEW ARTICLE


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## BRIEF REPORTS


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*751* Combination of 6-Mercaptopurine, Vincristine, Methotrexate, and Prednisone in Resistant or Relapsed Acute Nonlymphoid Leukemia

*755* Phase II Study of Continuous-Infusion Diaziquone in Relapsed/Refractory Acute Nonlymphocytic Leukemia

*759* Phase II Treatment of Central Nervous System Gliomas With High-Dose Etoposide and Autologous Bone Marrow Transplantation


## CLINICAL TRIALS SUMMARIES


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*765* Phase II Study of Esorubicin in Patients With Advanced Colorectal Carcinoma and No Prior Chemotherapy: An Illinois Cancer Council Trial

*767* Phase II Trial of Esorubicin in the Treatment of Metastatic Carcinoma of the Kidney: A Study of the Northern California Oncology Group

*769* Phase II Study of the Combination of Vinblastine Plus Cisplatin Administered by Continuous 120-Hour Infusion for Patients With Advanced Malignant Melanoma

*771* Phase II Study of Iv Menogaril in Patients With Malignant Melanoma: A National Cancer Institute of Canada Clinical Trials Group Study

*773* Phase II Evaluation of Aclarubicin in Refractory Multiple Myeloma: A Southeastern Cancer Study Group Trial

*775* Phase II Study of Oral Idarubicin in Patients With AIDS-Associated Kaposi's Sarcoma

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*779* 5-Fluorouracil and Cytarabine—Ineffective Treatment in Advanced Colorectal Carcinoma: A Southeastern Cancer Study Group Trial

*781* Phase II Study of Carmustine in Advanced Upper Aerodigestive Cancer

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*786* Pulmonary Toxicity of Lomustine

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*790* "Cisplatin Plus Etoposide as Second-Line Treatment in Advanced Ovarian Carcinoma"



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